Molting and Growth in Crayfish : A Review

D. E. Aiken and S. L. Waddy

Biological Station,
St. Andrews, N. B. E0G 2X0

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MOLTING AND GROWTH IN CRAYFISH: A REVIEW

by

D. E. Aiken and S. L. Waddy
Fish Aquaculture and Applied Physiology
Department of Fisheries and Oceans
Biological Station
St. Andrews, New Brunswick E0G 2X0
Canada

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ABSTRACT

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Crayfish increase their size by shedding their exoskeleton and expanding their underlying flexible integument. The rate at which they grow depends on the frequency of molting and the size increase at each molt. Temperature, photoperiod, nutrition, hydrological conditions, stress and reproductive state are some of the factors controlling molting and growth. However, temperature is the dominant parameter affecting growth. At the northern extreme of the distribution, the temperature is too low for molting in all but 2-3 mo in the summer. At the southern extreme, on the other hand, temperature may be too high for molting in the summer. Crayfish undergo complex biochemical and morphological changes in preparation for molt. The inner layers of the shell are dissolved and the minerals they contain are stored. The new cuticle is secreted under the old and rapid growth of the epidermis just before molt results in expansion folds and ripples underneath the rigid exoskeleton. This allows the newly molted animal to increase in size by ingesting and absorbing water to inflate the soft postmolt integument. Just before molt, the claw muscles are degraded to a fraction of their previous volume so that they can be withdrawn through the small upper leg segments. All of the complex processes involved in molt preparation and recovery are controlled by the endocrine system. Molting is controlled by two groups of hormones: molt inhibiting hormone that suppresses molting, and molting hormone that induces premolt and regulates molting.

RÉSUMÉ

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Les écrevisses augmentent leur taille par l'abandon de l'exosquelette et l'expansion du tégument extensible sous-jacent. Le rythme de croissance dépend de la fréquence de la mue et de l'augmentation de la taille à chaque mue. La température, la photopériode, la nutrition, les conditions hydrologiques, le stress et la phase reproductive constituent certains des facteurs qui régissent la mue et la croissance. La température est toutefois le principal paramètre qui influe sur la croissance. A l'extrême nord de l'aire de distribution, la température est trop basse pour la mue, sauf pendant deux ou trois mois de l'été. Par contre, à l'extrême sud, les températures peuvent être trop élevées pour que la mue s'opère l'été. Les écrevisses subissent des changements biochimiques et morphologiques complexes avant la mue. Les couches internes de la carapace se dissolvent et les minéraux présents sont préservés. La mouvelle cuticule est secrétée sous l'ancienne et, grâce à la croissance rapide de l'épiderme juste avant la mue, il se forme des plis et des sillons d'expansion au-dessous de l'exosquelette qui est rigide. L'animal qui a mué accroît alors sa taille en ingérant et en absorbant de l'eau pour gonfler le tégument mou de la postmue. Juste avant la mue, les muscles de la pince s'atrophient et n'atteignent plus qu'une fraction de leur volume antérieur de façon à pouvoir se rétracter par les petits segments de la patte supérieure. Tous les processus complexes qui entourent la préparation de la mue et la récupération sont contrôlés par le système endocrinien. La mue est régie par deux groupes d'hormones: l'hormone qui exerce une action d'inhibition et supprime la mue et l'hormone de la mue qui déclenche la prémue et régularise la mue.

INTRODUCTION

Survival, growth and reproduction are the trinity of existence for an organism. To ensure its continued success, a species must reproduce. To reproduce, it must grow. To accomplish either, it must survive.

This report is about the second element - growth. For a crustacean, growth means ecdysis, or molting - the casting off of the external skeleton to permit expansion of soft tissues and an increase in body size or volume.

A highly calcified crustacean expresses its growth differently than most other animals. Whereas cell division and protein synthesis cause continuous change in the external shape or volume of most animals, the dimensional increases of crayfish occur at intervals and are maximally out or phase with cell division and protein synthesis. In other words, a crustacean can grow in size only after it has completed a growth phase and has cast off its constraining exoskeleton. The business of doing so is an unbelievably complex and demanding exercise that insinuates most of the life processes of the animal.

'Molt' is simultaneously a general and a specific term, a concept and an event. The concept is the suite of physiological, biochemical, behavioral and anatomical changes involved in preparation for and recovery from ecdysis. The event is ecdysis itself, the actual shedding of the exoskeleton. Terms such as premolt, postmolt and intermolt are derived from the colloquial use of 'molt' to denote the shedding of the exoskeleton. However, words - like organisms - evolve with time. As molt evolved from an event to a concept (or even to a different event, as in its aborted synonymy with apolysis), the need for greater precision led to the substitution of proecdysis for premolt, and metecydsis or postecdysis for postmolt.

However, the old terms have endured in spite of the inherent confusion. Thus 'intermolt cycle' has become synonymous with 'molt cycle' even though the former was intended to exclude ecdysis (Drach 1939), and the various derivations of 'molt' are interpreted as a simple matter of context. The irony is that by substituting new words for old to reduce confusion, the confusion has multiplied.

The terms molt, postmolt, intermolt and premolt are entrenched and generally understood, and will be used in this report except where the possibility of confusion requires more precise terminology.

THE MOLT CYCLE

Crayfish, like most crustaceans, spend much of their lives either preparing for or recovering from ecdysis. Premolt induction and preparation for ecdysis are under the control of the endocrine system which in turn responds to internal (eg. nutritional state) and external cues (temperature, photoperiod, etc.). Premolt metabolic activities include limb regeneration, resorption and storage of cuticular components, deposition of new cuticle, histolysis of somatic muscle, selective water and ion absorption, lipid synthesis and biochemical shifts in metabolic pathways. All of these culminate in ecdysis, the shedding of the old exoskeleton.

After ecdysis, the crayfish assumes its new length and volume by actively absorbing water. The epidermis then regresses, additional cuticle is secreted and mineralized, the water is replaced by new tissue and metabolic reserves are gradually replenished. The crayfish may then move directly into preparation for the next molt or pause for an extended period (the 'diecdysis' and 'anecdysis', respectively, of Carlisle (1953)).

STAGES OF THE MOLT CYCLE

In progressing from one molt to the next, a crayfish passes through a series of well defined morphological and physiological states. Olmsted and Baumberger (1923) and Baumberger and Olmsted (1928) are credited with the first rational attempt at an intermolt staging system. They used (but did not necessarily coin) such descriptive terms as paper shell and pillans. However, Drach (1939, 1944) was the first to devise a staging system that had a measure of universality and recognized the truly cyclic nature of events. On the basis of changes in the integument, he divided the intermolt cycle (i.e. those events between one ecdysis and the next) into four periods (A, B, C, D) with numerous subdivisions.

Over the years, both this scheme and its terminology have been modified and adapted to different crustaceans. Stage E (ecdysis) was added (see Knowles and Carlisle 1956), making the designation 'molt cycle' more appropriate, and the terms premolt, proecdysis, postmolt, metecdysis, intermolt, diecdysis and anecdysis have been introduced. Where Drach had but 12 subdivisions for the entire molt cycle, up to 10 subdivisions now exist for stage D alone (Aiken 1973).

Detailed and generally accepted molt staging criteria are necessary for the characterization of physiological and biochemical events and for the comparison of those events between species. Various modifications of the staging system devised and subsequently refined by Drach (Drach and Tchernigovtzeff 1967) are now used in all critical studies of crustacean molting. These are briefly discussed in the following sections.

Stage A

Stage A begins as soon as the animal is free of the exuvia. The body of the newly emerged crayfish is flaccid and wrinkled, but ingestion and absorption of water to at least 12% of the body weight (Andrews 1967) soon expands the animal to its new volume, generally some 50% greater than before ecdysis. Strategic portions of the new cuticle - tips of chelipeds, cutting edges of mandibles and maxillipeds - are mineralized from reserves stored in the hepatopancreas or in stomach concretions called gastroliths ('crab's eyes'). At that point, the paper-shell crayfish is able to begin foraging.

Drach (1939) divided stage A into A_1 and A_2 and defined three criteria to separate the two: the onset of endocuticle secretion, onset of calcification and the presence of an amber-colored substance in the pre-exuvial layers. Stevenson (1968) felt it unlikely that all three criteria would appear simultaneously in all species and suggested (Stevenson 1968, 1985) that the onset of

endocuticle secretion be adopted as the universal criterion for onset of stage A_2. Others have eliminated stage A_2 and transferred its critera to stage B (see following section).

Stage B

There are no universal criteria to differentiate stages A_2 and B. Stevenson (1968, 1985) separated the two by degree of shell hardness (Table 1), but this is arbitrary and varies with individuals and species. Others (Keller and Adelung 1970; Skinner 1985b; Travis 1965) have eliminated stage A_2 and used endocuticle deposition as the criterion for onset of stage B (Van Herp and Bellon-Humbert 1978) (Table 2). We prefer this solution because it provides a firm demarcation between stages A and B and can be used with all species.

Stage C

Stage B ends when chemical changes in the pre-exuvial layers (epicuticle, exocuticle) are completed (Drach 1939). Shell regidity is a more convenient index to use, and in Orconectes sanborni stage C begins when the postorbital ridge and cervical groove change from flexible to rigid, 1-3 d after onset of stage B (Stevenson 1968) (Table 1). The endocuticle then contains 3-5 laminae, but carapace sections are otherwise identical to sections in stage B. Stage C_2 can be arbitrarily determined from increasing rigidity of the exoskeleton, but there are not universal criteria for this stage because of the variation between species.

Table 1. Molt staging based on shell regidity in crayfish.

Molt stage	Astacus leptodactylus[1]	Astacus leptodactylus[2]	Orconectes sanborni[3]
A_1	Soft pereiopods		Cuticle soft, slippery.
A_2	Pereiopods more rigid		Cuticle like parchment.
B_1	Propodus, merus flexible		Postorbital groove of carapace brittle, but bends under finger pressure.
B_2	Propodus, merus brittle		
C_1	Flexible shell body	Pleurites flexible until end of stage C_1	Postorbital ridge and cervical groove change from flexible to rigid.
C_2	Rigid shell body	Shell fully hardened	Only the gastric region and areola are flexible.
C_3	Hard shell body		Carapace hardened fully. Some areas still flexible, but remain so.
C_4	-	-	-
D_0	-	-	-
D_1	-	-	-
D_2	Epidermal sutures flexible		
D_3	Epidermal sutures soft	Epidermal sutures soften	Epimeral suture breaks under finger pressure.
D_4	Opening of thoracoabdominal juncture		Epimeral suture opens.

[1]Van Herp and Bellon-Humbert 1978; [2]Vranckx and Durlist 1978; [3]Stevenson et al. 1968.

Table 2. Variation in timing of cuticular events during the molt cycle of four species of crayfish.

	Orconectes limosus[a]	O. sanborni[b]	O. virilis[c]	Astacus leptodactylus[d]
Endocuticle secretion begins	B	A_2	B	B
Endocuticle complete		C_4'	C_3	
Membranous layer secretion		C_4	C_3	C_3
Membranous layer complete		C_4	C_4	C_4
Apolysis	D_1	D_0	D_0	D_0
Epicuticle secreted	D_2'	D_2	D_2	D_0
Exocuticle secreted	D_2''	D_2	D_2	D_2
Resorption of old cuticle	D_3	D_3	D_3	

[a]Keller and Adelung (1970) - branchiostegite; [b]Stevenson (1968) - gastric region of carapace; [c]Travis (1962, 1965) - branchial exoskeleton; [d]Van Harp and Bellon-Humbert (1978).

O. sanborni enter stage C_2 about 1.5-2 d after the onset of stage C_1. In stage C_2, 4-6 laminae are present in carapace endocuticle, and the branchial region, gastric region and areola of the carapace are the only flexible areas (Stevenson 1968).

Stage C_3 can start as little as 2 d after the onset of C_2 in O. sanborni. C_3 is the final stage of postmolt, or metecdysis and, by the end of this stage, the integument has attained maximum rigidity (the branchial region of the carapace will often retain a considerable degree of flexibility). Stevenson (1968) was of the opinion that a person with experience could determine the point of maximum rigidity by external examination.

Stage C_4 commences with deposition of the membranous layer (Drach 1939). Drach distinguished between onset of membranous layer formation (C_4') and completion of the membranous layer (C_4). A similar designation was used by Stevenson (1968). In some crustaceans, the membranous layer can be identified by the 'broken cuticle test' in which a broken piece of cuticle from a stage C_4 animal will be held together by the membranous layer (Drach 1939; Drach and Tchernigovtzeff 1967). This test does not work with some crayfish because the inner endocuticle is flexible and will hold together even before the membranous layer has formed (Stevenson 1968).

Stage D

Stage D is premolt, or proecdysis, the physiologically significant period during which the animal is preparing for the next molt. Pore canals are severed, reserves are accumulated and the two pre-exuvial layers of the new cuticle are synthesized (Fig. 1). Unlike some of the earlier stages, each subdivision of Stage D can be recognized by a distinct change in integumental morphology (Drach and Tchernigovtzeff 1967):

D_0 - retraction of the epidermis from the old cuticle (apolysis)
D_1 - invagination of setal matrices
D_2 - secretion of the pre-exuvial layers
D_3 - resorption along epimeral sutures
D_4 - opening of the epimeral sutures.

In Procambarus clarkii, premolt accounts for more than 50% of the molt cycle (Hunner and Avault 1976a) and in Faxonella, stage D_0 alone may occupy 50-70% of the cycle (Rao et al. 1977). As in Homarus (Aiken 1973), stage D_0 in many crayfish is subject to prolonged periods of premolt inhibition (anecdysis), and it has been suggested that D_0 might be more appropriately considered the final stage of C_4 (Huner and Avault 1976a; Rao et al. 1977; Vranckx and Durliat 1978).

The most reliable indicator of onset of D_0 is epidermal retraction (apolysis) in the tips of flattened appendages. Onset of gastrolith formation is occasionally used, but gastrolith development is too variable to be a reliable indicator (Rao et al. 1977; Stevenson et al. 1968). This variation occurs in processes other than gastrolith development, and it can cause confusion when results from one study are compared with another. For example, in a recent review, Skinner (1985b) assigned apolysis to stage D_1. Apolysis is normally assigned to the onset of stage D_0 (Drach and Tchernigovtzeff 1967). In Homarus, Astacus and Orconectes, apolysis is clearly one of the first events of stage D_0 (Aiken 1973; Stevenson 1972; Vranckx and Durliat 1978). Why then did Skinner assign it to stage D_1?

The information reported by Skinner (1985b) appears to have been drawn from her earlier work (Skinner 1962) on Gecarcinus in which integumentary changes were followed throughout the molt cycle in a section of branchiostegite. In the branchiostegite, apolysis occurred in stage D_1. In the tips of Homarus pleopods, apolysis always precedes or coincides with onset of gastrolith calcification (stage D_0), but in the branchiostegite and meropodite, apolysis does not occur until the animal is in stage D_1 (Table 3). Thus in Homarus (and

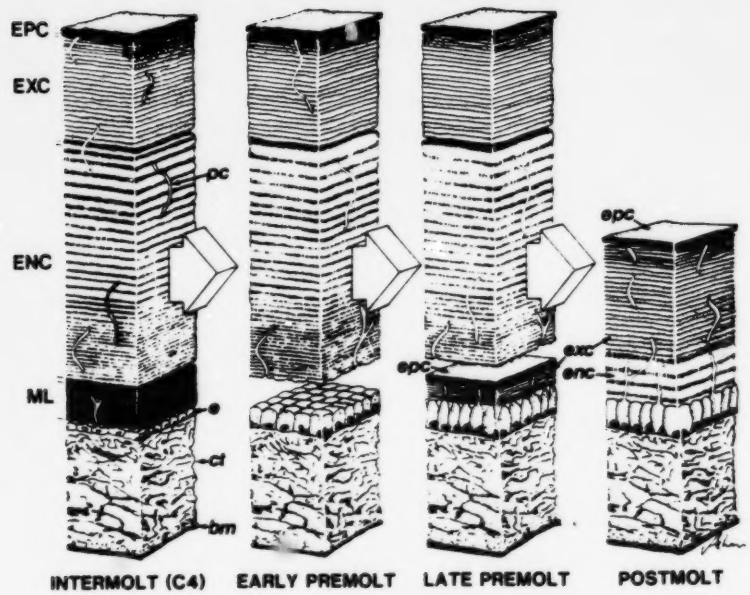

Fig. 1. Changes in the structure of the integument during the molt cycle. EPC, epicuticle; EXC, exocuticle; ENC, endocuticle; ML, membranous layer (the lower case labels identify equivalent layers of the new cuticle); e, epidermis; ct, connective tissue; bm, basement membrane; pc, pore canals.

Table 3. Variation in time of occurrence of selected premolt events in Homarus americanus.

Molt stage		D_0'	D_0''		D_0'''	D_1'	D_1''	D_1'''	D_2'	D_2''	D_3
Pleopod stage*		1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Pleopod	Ep	X									
	Apo		X	X	X						
	ML		X	X	X						
	Seto					X	X	X	X	X	X
	Epic								X	X	X
	Exoc									X	X
Carapace	Ep				X						
	Apo					X	X				
	ML					X	X	X			
	Epic							X	X	X	X
	Exoc								X	X	X
Merus	Apo					X	X				
	ML					X	X	X			
	Epic							X	X	X	X
	Exoc								X	X	X
Gastrolith	Ep	X	X								
	Calc		X	X	X	X	X	X	X		
	Epic									X	X
	Exoc										X

Apo - apolysis; Calc - calcium deposition; Ep - epidermal activity; Epic - new epicuticle present; Exoc - new exocuticle present; ML - membranous layer degeneration; Seto - setal development. *Stages as designated in Aiken (1973).

probably also in Gecarcinus) the variation in different parts of the integument is such that some areas can be one full molt stage behind events elsewhere in the animal.

The point, as Stevenson et al. (1968) noted, is that premolt development occurs at varying times and rates in different anatomical regions within the same animal. Since it is the physiological state of the animal that is important, not the state of a particular piece of tissue within the animal, this variation should be borne in mind.

Stage E

Although not a part of Drach's original description of the intermolt cycle (pace Knowles and Carlisle 1956), stage E is now generally included.

The first detailed description of ecdysis in a crayfish appears to be that of Réaumur (1712) for Astacus, more than 270 yr ago. At that time, ecdysis was thought to be a brief interruption in the life of a decapod, but we now recognize that preparation for and recovery from ecdysis occupies more than half of the animal's time and metabolic activity.

Ecdysis is often divided into a passive and an active phase. During the passive phase, the epimeral sutures are decalcified but the animal remains mobile. Water is ingested or absorbed and redistributed, and as hydrostatic pressure increases, the thoracoabdominal membrane begins to bulge. The animal, if deprived of shelter or privacy, may become agitated, moving about with chelipeds extended forward and, if conditions for ecdysis are unfavorable, the passive phase can be prolonged.

The active phase of ecdysis commences when hydrostatic pressure is sufficient to rupture the dorsal membrane between carapace and abdomen. The animal then loses mobility and rolls onto its side (Fig. 2). The carapace pivots up and forward, and the cephalic, thoracic and abdominal appendages and gills are withdrawn. The exuvia is a remarkably life-like replica of the living animal, complete with eye facets, gill filaments and the lining of the fore and hind gut.

The major chelate appendages (first pereiopods) present a special problem for the molting animal. The muscle of the intermolt propodite is too large to pass through the segments of the upper leg without causing serious injury at ecdysis. To facilitate withdrawal, two remarkable events occur during premolt: the muscle mass of the propodite is progressively degraded by a calcium-dependent proteinase until it is just a fraction of its stage C mass (Mykles and Skinner 1982), and the upper segments of the leg are extensively decalcified. This combination, plus lubrication by 'molting fluid', ensures easy withdrawal of the limb at molt.

Because of danger from predators, normal awareness and mobility must be maintained by the animal even while neuromuscular control is being transferred from the old cuticle to the new one forming underneath. This transition is accomplished so efficiently that a crayfish is able to maintain a high level of coordinated activity right through the passive phase of ecdysis. Immobility and vulnerability occur only after the thoracoabdominal

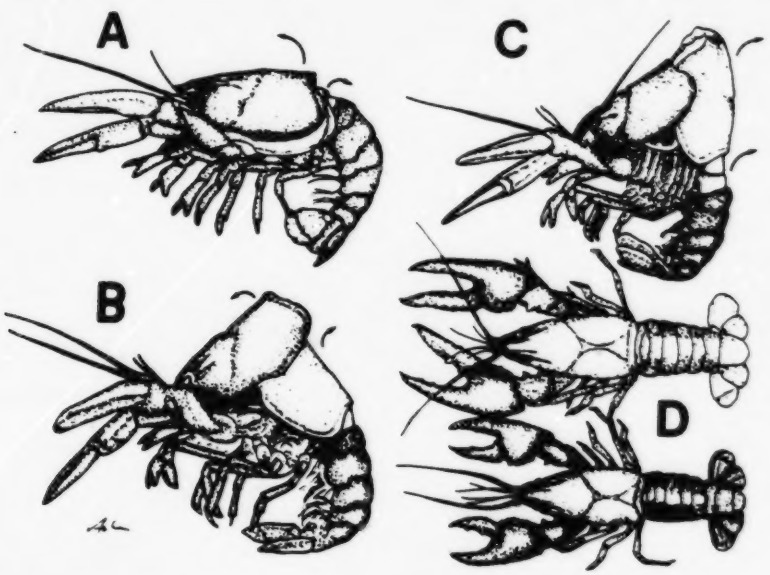

Fig. 2. The molting process (ecdysis) in crayfish. A, the membrane between the thorax and abdomen ruptures dorsally, the animal lies on its side and the old carapace swings up and forward. B, anterior appendages are withdrawn as the animal pulls back from the old exoskeleton. C, the head emerges from the exuvia and the chelipeds, walking legs, gills and abdomen follow. With a flip of the tail the animal is free. Water is absorbed and redistributed to expand the integument (D), transforming the newly molted animal (above) to a larger size than the exuvia from which it emerged (below).

membrane ruptures and the old carapace begins to lift free. Normally, no more than 10 min elapse from that moment until the flaccid animal emerges from the exuvia.

Alternate Terminology

The generally accepted molt cycle subdivisions used above have been supplemented (and occasionally supplanted) by varied and overlapping terminology. Postmolt and its synonym metecdysis encompass stages A, B and C_1 through C_3. Intermolt usually refers to stage C_4, although it is not universally appreciated (see Drach and Tchernogovtzeff 1967). Diecdysis is an intermolt that proceeds directly to the next premolt. If passage through premolt is delayed for a considerable period, it is generally referred to as anecdysis (although anecdysis has also been used in synonymy with intermolt - compare Skinner (1985b) with Carlisle (1953)). Premolt and proecdysis are alternate terms for stage D.

MOLT STAGING TECHNIQUES

For studies that involve the molt cycle, a universally applicable staging system is a necessity. Two types of staging systems are in use: those based on changes in shell color and hardness, and those based on changes in developing setae (setogenesis).

Shell Color and Hardness

In highly calcified crustaceans, molt stage can be estimated from shell hardness or color. However, rate and degree of mineralization - and therefore apparent molt stage - can be altered by diet, temperature, pH and other factors, so external criteria are of little value in physiological studies. The various degrees of shell rigidity and the corresponding molt stages for two species of crayfish are summarized in Table 1.

Crayfish become progressively darker in color as they approach a molt, and the new cuticle can be seen through the membranes of the old (Huner and Barr 1984; Stevenson et al. 1968). Presence of new cuticle can also be determined by breaking off the tip of a chelate appendage.

Substages of premolt can also be estimated from the growth of regenerating limb buds (Stevenson et al. 1968; Stevenson and Henry 1971) or - by sacrifice or X-ray - size of the gastroliths (Connell 1970; McWhinnie 1962; Rao et al. 1977; Stevenson et al. 1968). In very young Procambarus clarkii, the cuticle is so thin that the gastroliths are visible through the shell (Huner and Barr 1984).

Setogenesis

Setal staging criteria have been described for several species of crayfish, and uropods, telson, scaphocerites and scaphognathites have been used (Table 4). The uropods and telson are often opaque and may have a secondary or even a tertiary row of setae, which makes staging more difficult. Scaphocerites and scaphognathites are usually clipped for sampling, and this severely limits the opportunity to monitor changes within individuals over time.

The best decapod for setogenic molt staging appears to be the American lobster, an animal whose eight major pleopods each has a pair of large, flattened, transparent blades, making 16 successive samples possible from an individual (Aiken 1973). This fact, plus the lobster's relatively slow progression through the molt stages, permits premolt setogenic changes to be followed in individual animals. This has not been possible with crayfish because of the limited number of transparent appendages and rapid development through the molt stages. However, the evidence suggests the setogenic process in the American lobster is similar to that in many crayfish, which means that Homarus can be used to study and explain setogenic changes that are difficult to observe and follow in crayfish (Fig. 3).

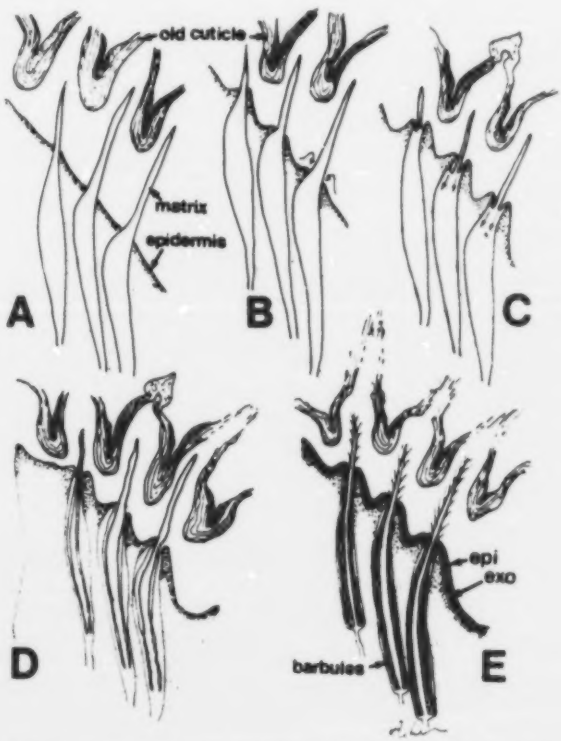

Fig. 3. Steps in the formation of new setae in the tips of flattened appendages. A, epidermis retracts from cuticle (stage D_0). B, C, epidermis invaginates at sites of new setae (stage D_1). D, invaginating epidermis visible as new setal shafts. E, new barbules apparent on well developed setal shafts (stage D_2). epi, epicuticle; exo, exocuticle.

Of those crayfish for which there are reported studies, Orconectes sanbornii, O. obscurus, Astacus leptodactylus and Procambarus clarkii follow the Homarus plan. Differences that exist in published descriptions appear to be due to difficulty in observation rather than differing methods of setal formation. The exception appears to be Parastacoides tasmanicus (Mills and Lake 1975).

Detailed information exists for Astacus leptodactylus in two separate studies (Van Herp and Bellon-Humbert 1978; Vranckx and Durliat 1978). Their photographs and descriptions leave little doubt that the sequence and pattern of setal

Table 4. Criteria used for molt staging from setal development in the appendages of four species of crayfish.

Molt stage	*Parastacoides tasmanicus*[a] (Uropods and telson)	*Astacus leptodactylus*[b] (Scaphognathite)	*Astacus leptodactylus*[c] (Uropods-Scaphognathite)	*Orconectes sanborni*[d] (Uropods)	*Procambarus clarkii*[e] (Uropods)
A	No internal matrix in setae	Fibrillar elements in setae	Uropods display distinct setal matrices with well structured canals		Moving fluid visible in new setal shafts of live crayfish
B	Internal matrix develops in setae	Nerve fibers visible in setae			No moving fluid visible in setal shafts of live crayfish
C	Internal cone develops at apex of internal matrix	Retraction of epidermis into setal articulation	Development of light zone bordered by chromatophores at end of C		Inner cones above bases of new setae well developed
D_0	Epidermal retraction	Epidermal retraction	Retraction difficult to because of uropod thickness. Inner fibrillar structures visible in setal extensions	Epidermal retraction	Epidermal retraction
D_1'	New setae develop and invaginate into uropod and telson	Formation of invagination fold of new setae	Fibrillar structures reach 0.5 distance from edge of shell to first spine		Retracted epidermis distinct but no evidence of extensive invagination
D_1''	Setae continue to invaginate toward the translucent zone	Formation of new setal matrix	Invaginated fibrillar structures extend 0.75 distance from edge of shell to first spine	Cuticle of new setae begins to form	Evidence of invagination visible distal to margin of retracted epidermis
D_1'''	Maximum invagination. Barbules develop on apex of setae	Barbules form on new setae	Setae clearly visible and fully invaginated. Barbules noticeable	Barbules appear on setae	Invagination complete. New setae as tubes within tubes. Proximal ends indistinct. Barbules on distal end of setae
D_2	No further development	Beginning of setal extrusion	Invaginated setae darker and more opaque	No change in setae	Barbules on central region of shaft. Setal bases shaped like inverted 'V'
D_3	Barbules completed, setal evagination begins	Setal evagination complete	Folds develop on integument of appendage	No change in setae	Setal bases distinctly rounded

[a]Mills and Lake 1975; [b]Van Herp and Bellon-Humbert 1978; [c]Vranckx and Durliat 1978; [d]Stevenson et al. 1968; [e]Huner and Barr 1984.

development in the crayfish is identical to what occurs in Homarus.

The stage criteria of Van Herp and Bellon-Humbert are similar to those of Vranckx and Durliat through Stage D_1'', but deviate at D_1''', where Van Herp and Bellon-Humbert describe the setal wall as fully constructed and armed with barbules. This sounds more like stage D_2, and their figures 7 and 8 reinforce this.

It must be remembered that setal staging and molt staging are different. Drach (1939) based his intermolt stages on changes in the integument. As already noted, onset of a given integumentary process varies from one area of the body to another. Regarding stage D_2, Drach (1939) mentioned the carapace and the terminal segment of a walking leg. Therefore, observed setal changes should be correlated with actual histological evidence from the carapace or dactylopodite before a stage is assigned. There was no histological correlation in either of the two studies on A. leptodactylus, but in Homarus, where setal changes have been correlated histologically with changes in the carapace integument, completion of the setal wall, obvious barbule formation and prominent proximal ends of invaginated setae are characteristic of stage D_2, not D_1'''. Similarly, the start of setal evagination, which Van Herp and Bellon-Humbert assigned to D_2-D_3 (their figure 8), can be seen to occur exclusively in D_3 (and D_4, if one uses that stage designation).

The criteria for stages D_1 and D_2 are not straightforward, even when Drach's basic system and subsequent revisions are used. This has to do with apparent versus real changes, and the variation in onset and rate of cuticle synthesis in different anatomical locations. This problem has been reviewed in detail (Aiken 1973). Briefly, D_2''' is reached when the new seta has reached maximum invagination but the proximal ends of the setal shafts are not clearly defined. Stage D_2' is attained when the proximal ends of the setae are well defined but bifurcate (as opposed to blunt, or squared-off). Barbules may be present in both stages. These criteria are given in Table 5.

Table 5. Setal development stages that can be observed in flattened, transparent appendages during intermolt stages C and D.

Molt stage	Setal stage	Description
C_4'		Membranous layer forming in carapace integument but not yet complete.
C_4''		Carapace integument complete - so-called 'intermolt'.
D_0'	1.0	Clear, amber or double-bordered region formed at tip of appendage. Chromatophores may show signs of reorganization but there is no epidermal retraction.
D_0''	1.5	Epidermis retracting from cuticle at tip of appendage. Epidermis may have double-bordered appearance.
	2.0	Epidermal line clearly formed and retraction obvious.
D_0'''	2.5	Maximum epidermal retraction attained.
D_1'	3.0	Invagination papilla forms on epidermis at site of future seta. Epidermis assuming scalloped appearance.
D_1''	3.5	Invagination papilla clearly formed but shafts of new setae not well defined.
D_1'''	4.0	Shafts of developing setae visible but proximal ends not well defined. Setal shafts have invaginated to maximum length.
D_2'	4.5	Shafts of setae visible full length but proximal ends are bifurcate instead of blunt. Barbules becoming visible on shafts of setae.
D_2''	5.0	Shafts of developing setae thick, proximal ends blunt.
D_3	5.5	Shafts of setae thick and dark, proximal ends blunt.

There are two basic reasons for determining molt stages: to facilitate interpretation of experiments within a species, and to facilitate comparison of similar studies on different species. If everyone used different molt stage criteria, neither of these objectives could be attained. Stevenson (1972) therefore advocated the acceptance of universal landmarks for various molt stages. Stage D_2, for example, would commence with the secretion of exocuticle in the carapace integument, irrespective of what was happening elsewhere in the body of the animal. Once pleopod changes were histologically correlated with the landmark criteria, direct comparisons could be made within and between species.

The problem is there is no a priori reason to assume that onset of exocuticle synthesis in the carapace is representative of the same physiological state in all species, and it is the physiological state that is important, not the fact of cuticle synthesis at some arbitrary point in the carapace integument. Apolysis signifies an important change in the physiological state of the animal - an alteration in the hormonal milieu - but this event can be detected much sooner in the integument of flattened appendages than in the integument of the carapace. In other words, if the carapace integument is used as the standard, onset of one of the major physiological events of the intermolt cycle will be missed by at least a full molt stage.

Thus, the principle of universality may be a myth, and the practice of relating precise setal stages back to Drach's classical molt staging system may be a mistake. From an experimental point of view, we do not really care when the carapace exocuticle is laid down as long as it is universally representative of a common physiological state. Unfortunately, there is no reason to assume that it is. Thus, we are taking an event that can be observed directly (setal development) and reporting it in terms of an unseen event of questionable physiological significance (Drach's integumentary intermolt stage), thereby introducing error and uncertainty.

Setal development can be observed with varying degrees of success in many decapods, and the process involves a sequence of events that can be easily catalogued. Instead of arguing whether maximum setal invagination occurs in molt stage D_1'', D_1''' or D_2, we should simply assign a specific setogenic stage to the event and not worry about the molt stage. The setal stage can be observed directly and, although there may be some slight variation among and between appendages and species, the precision and repeatability from setogenic staging will be much greater than can be obtained by converting the setal stages to the classical molt stage designations.

A setogenic staging system was devised for Homarus (Aiken 1973) and it appears equally applicable to Astacus, Orconectes and Procambarus. These 10 substages, modified to encompass more recent information, are given in Table 5 and Fig. 4. We advocate the development of setogenic stages for as many crayfish species as possible, and the utilization of these (instead of their assumed molt stage analogs) in the reporting of results.

Setal staging has already made a significant contribution to the question of anecdysis. In the classical system, anecdysis was considered a part of stage C_4, but setal staging has showed that early epidermal activity and apolysis are characteristic of animals in anecdysis (Aiken 1973; Vranckx and Durliat 1978). This event is not obvious in fixed sections of the carapace integument. If apolysis is the landmark for stage D_0 (Drach and Tchernigovtzeff 1967), then anecdysis occurs in premolt, not stage C_4. Recognizing this, Vranckx and Durliat (1978) suggested that what we now call early D_0 should be considered the final stage of C_4. We have resisted the temptation to do so in Table 5 because the change would shift part of stage D_0 into stage C. The problem is avoided by simply using setal stages to describe the physiological/ morphological condition of the animal.

Finally, there is the question of how the setae form. According to Mills and Lake (1975), there are two different methods of setal formation in the Decapoda: by invagination of the retracted epidermis (Natantia, Brachyura), and by formation within a 'setal organ' (Macura, Anomura). Vranckx and Durliat (1978) rejected this, feeling there are simply two types of 'quiescent matrices' that give the appearance of either a setal organ (contracted form of matrix that does not retract from the lumen of the seta during the intermolt period), or an epithelial cylinder inside the tissue (elongated form of the matrix that does retract from the old seta). The setal organ, according to these authors, is not a special structure but simply the 'preception (sic) of nuclei and cell bodies of the matrix which leave the epithelium level and move inwards into the hypodermal tissue....'

Mills and Lake (1975) have overlooked the fact that setae in the Macrura also form by invagination of the retracted epidermis (Fig. 3), but this does not, in itself, negate their premise that Natantia and Brachyura form setae in a different way from the Macrura. Whether the setal organ is an artifact, as Vranckx and Durliat suggest, remains to be demonstrated. Suffice to say that the structures depicted in Fig. 2 of Aiken (1973) show evidence of integrity and hemolymph supply in histological section as well as in the freshly dissected state.

BIOCHEMICAL CHANGES

The literature contains an impressive amount of information on the biochemical changes that occur during the molt cycle. However, the subject has been adequately reviewed (Andrews 1967; Chang and O'Connor 1983; O'Connor and Gilbert 1968; Waterman 1960), so only an overview is required here.

Crayfish prepare for their next molt by accumulating organic reserves such as phosphate, glycogen, lipid and protein in the hepatopancreas. The hexosemonophosphate pathway provides a biochemical basis for increased protein and lipid synthesis during intermolt (McWhinnie and Corkill 1964), and lipids, in the form of fatty acids and glycerol, comprise the major portion of the stored reserves. Much of this lipid is subsequently converted to glucose for use in chitin formation during premolt (Passano 1960), resulting in a significant increase in blood glucose level during premolt and a corresponding decrease during postmolt (Chang and O'Connor 1983).

Hepatopancreas activity varies with season and molt stage (McWhinnie and Kirchenberg 1962). Metabolism is elevated during pre- and postmolt, probably because of the conversion and release of

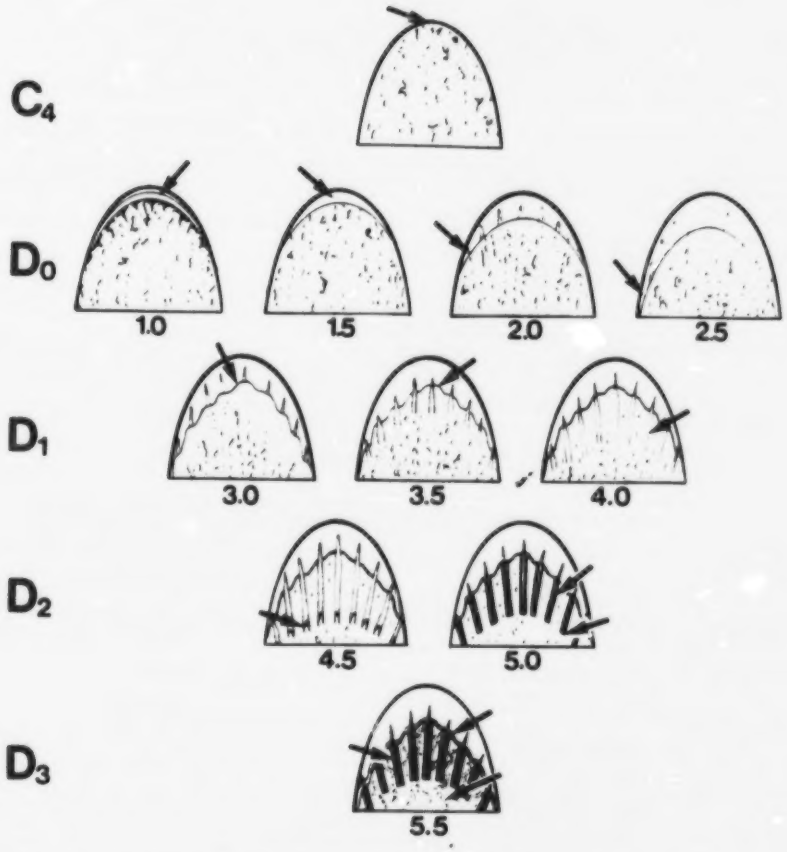

Fig. 4. Setogenic stages. In animals with flattened transparent appendages, 10 stages can be assigned to the development of new setae. These setogenic stages permit a greater degree of precision and repeatability than can be obtained by using classical molt staging criteria.

organic reserves (Passano 1960; Vonk 1960). Tissue metabolism can elevate oxygen consumption by as much as 1900% during premolt (Skinner 1962).

Protein is stored in the hepatopancreas during both intermolt and premolt, and reaches maximum levels during stages C_4 and D_2-D_3 (Durliat and Vranckx 1982). Sugar, fat, total calcium and total protein levels in the hemolymph also increase during premolt (Florkin 1960), and there is progressive degradation of the old cuticle and the large muscle mass of the chelipeds (Skinner 1985b). As the cuticle is degraded, the hemolymph takes on a characteristic pink coloration due to accumulating amino acids and the pigment astaxanthin.

THE INTEGUMENT

The decapod integument consists of a rigid cuticle underlain by cellular epidermis. The four distinct layers of the cuticle are modified and generally chitinized proteinaceous material secreted by the epidermal cells (Richards 1951). These four layers of the cuticle are often considered to be a nonliving shell protecting the living tissue beneath. However, cytoplasmic extensions of the epidermal cells traverse the cuticle to the epicuticle through the pore canals. These pore canals are extremely abundant, 50-90 emanating from each epidermal cell. Travis (1963) estimated their numbers in the branchial exoskeleton of Orconectes to be approximately 4 million/mm² of cuticle and, from this, Halcrow (1976) calculated that no part of the cuticle was more than 0.25 µm from epidermal cytoplasm.

The pore canals have a helical or twisted ribbon morphology with a helical pitch that is equal to the lamella period (Drach 1939). They remain in continuity with the cuticle-secreting cells of the epidermis until apolysis, indicating the cuticle is able to receive cell products throughout stages A to C_3 (Green and Neff 1972). These facts suggest the cuticle should be considered living tissue (Roer and Dillaman 1984).

The literature contains a confusing assortment of terms for the four layers of the decapod cuticle (Aiken 1980, for review) but the nomenclature utilized by Richards (1951) for insects is now

widely used for crustaceans (Aiken 1980; Travis 1963, 1965; Skinner 1985a, b; Roer and Dillaman 1984; Green and Neff 1972; but see Giraud-Guille 1984a and Stevenson 1985). These layers, moving outward from the epidermis, are membranous layer, endocuticle, exocuticle and epicuticle (Fig. 1). The first two are formed after ecdysis and are therefore referred to as postexuvial layers.

STRUCTURE

Epicuticle

The epicuticle is the outermost cuticular layer, a thin 7 μm in the crayfish Orconectes virilis. In this species, the epicuticle lacks chitin and calcium and is hardened after ecdysis by the compounding of organic components (Travis 1965). In most crayfish species, the epicuticle is deposited in stage D_2 (Travis 1965; Keller and Adelung 1970; Stevenson 1968) and is completed after ecdysis, but Van Herp and Bellon-Humbert (1978) assigned it to D_0 in Astacus (Table 2).

The crayfish epicuticle has an inner, relatively thick lipoprotein layer and a very thin outer layer (Travis 1965). Ultrastructural studies have revealed at least six distinct zones in the epicuticle of the fiddler crab (Green and Neff 1972), and Arsenault et al. (1984) identified four zones in the American lobster. The crayfish epicuticle was once thought to be non-lamellar (Travis 1965; Kummel et al. 1970), but ultrastructural studies have since revealed a lamellar structure (Nyhlén 1975).

The decapod epicuticle is hardened by phenolic tanning before and after ecdysis and, in some species, it may be further hardened by calcification during postmolt. Drach's (1939) conclusion that the decapod epicuticle is calcified was supported by the work of Arsenault et al. (1984) on Homarus, but Travis (1965) and Travis and Friberg (1963) were unable to identify calcium in the epicuticle of Orconectes virilis.

Epicuticle calcification apparently depends on the presence of pore canals. In the crab Cancer the pore canals penetrate the basal layer of the epicuticle and mineralization occurs in the canals (Hegdahl et al. 1977). Likewise, in Homarus, the pore canals penetrate half way through the epicuticle and calcium deposits are found there (Arsenault et al. 1984). In the fiddler crab, Uca, on the other hand, the pore canals do not enter the epicuticle itself but may extend into the exocuticular regions between the epicuticular 'tailings' (Green and Neff 1972) (Fig. 5), an arrangement that could easily give a false impression of epicuticular calcification. Most studies point to a clear relationship between the termination of pore canals and the termination of calcification. The exception appears to be O. virilis. Travis (1965) stated that the epicuticle of this crayfish is crossed by pore canals, but calcium was not detected by histochemistry, microradiography or X-ray diffraction (Travis 1965; Travis and Friberg 1963).

Exocuticle

The exocuticle, also a preexuvial layer, underlies the epicuticle but is much thicker. In Orconectes, it is composed of approximately 12 lamellae that vary from 3-8 μm with a total thickness of approximately 60 μm (Travis 1965). The exocuticle is constructed of chitin microfibrils

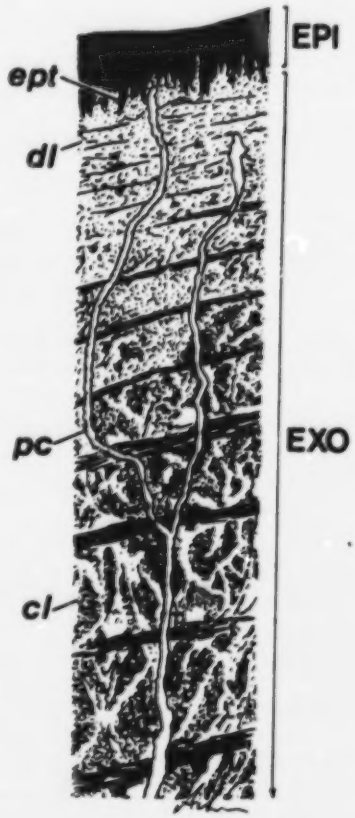

Fig. 5. Relationship between the pore canals of the exocuticle and the 'tailings' of the epicuticle of Uca. cl - continuous lamella; dl - discontinuous lamella; ept - epicuticular tailing; pc - pore canal; EPI - epicuticle; EXO - exocuticle (from micrographs in Green and Neff 1972).

embedded in a protein matrix and arranged in a helicoid pattern not unlike the glass-fiber plastic matrix used in modern boat hull construction (Neville 1984; Roer and Dillaman 1984, for reviews). The protein concentration in this layer is twice that of the other cuticular layers, and about 45% of the organic component is chitin (Welinder 1975a, b).

The exocuticle is lamellated and the horizontal bands are tied together by vertical or oblique fibrils that intermesh with fibrils of the horizontal bands (Neville 1984). It has been suggested that the vertical lamellae are optical artifacts (see Green and Neff 1972), but SEM studies have established that the chitin-protein fibers form numerous lamellae connected by vertical lamellae (Mutvei 1974) that are crossed by tegumental ducts and pore canals. The pore canals, containing cytoplasmic extensions, conduct calcium and other materials to the epi- and exocuticle for tanning and hardening after ecdysis (Travis 1963, 1965).

The exocuticle is hardened by quinone tanning and calcification by mineral crystals situated between the fibers (Giraud-Guille 1984b; Travis 1965).

Endocuticle

The endocuticle, one of the two postexuvial layers, consists of an outer thick-lamina zone and an inner thin-lamina zone. In the crayfish, the endocuticle is usually the thickest layer of the cuticle (up to 160 μm). In Orconectes, it consists of approximately 10 broadly spaced lamellae varying in thickness from 1-16 μm (Travis 1965). It is the most heavily calcified layer of the cuticle and, like the exocuticle, is traversed by pore canals and tegumental ducts. It differs from the exocuticle in not being tanned. Hardening of the endocuticle is solely by calcification, which occurs in conjunction with lamella formation (Travis and Friberg 1963).

Yano (1972) reported a distinct wavy boundary between the exocuticle and endocuticle, but Green and Neff (1972) considered the transition between the two layers to be abrupt. The endocuticle, like the exocuticle, is composed of horizontal lamellae of chitin-protein fibers with continually changing orientation (Green and Neff 1972). About 87% of the organic component of the endocuticle is chitin, almost twice that of the exocuticle (Welinder 1975b).

Mineral in the outer three layers of the crayfish consists of calcium carbonate in the form of calcite or poorly crystalline, amorphous calcium carbonate (Travis 1963). In the Cambaridae, it constitutes 20-26% of the dry weight of the carapace (Huner and Lindqvist 1985) and 80% of the total material deposited during postmolt (Welinder 1975b).

Membranous Layer

The membranous layer is the innermost layer of the cuticle and is in direct contact with the epidermis. It is a relatively thin 10 μm in Orconectes (Travis 1965) and comprises only 6.4% of the total weight of the cuticle (Welinder 1975a). The membranous layer is the last to form (stage C_4') and the first to be broken down (stage D_0). In Orconectes, it is composed of approximately 16 uncalcified and finely spaced lamellae (average 0.5-0.7 μm thick) that are traversed by pore canals (Kummel et al. 1970; Travis 1965).

Epidermis

The epidermis is in contact with the cuticle and is responsible for the partial digestion of the old exoskeleton and the synthesis, secretion and mineralization of the new. It is a monolayer that varies from squamous to columnar as it undergoes cycles of recrudescence and retrogression correlated with the molt cycle (Green and Neff 1972).

Underneath the epidermis is the subepidermal connective tissue consisting of reserve cells, blood sinuses with hemocytes, lipoprotein cells and pigment cells (Travis 1965). A basement membrane lies between the connective tissue and the underlying hemocoele.

CYCLIC CHANGES

Intermolt Integument

'Intermolt', as used here, refers to the period of time between the completion of the membranous layer early in stage C_4 and the start of dissolution of the integument at the end of stage C_4. In the system devised and modified by Drach this is stage C_4, between C_4' and the start of D_0.

During C_4, the epidermis is in its most reduced state and is in close contact with the membranous layer of the overlying exoskeleton. The epidermal cells are only 9 μm high in Orconectes, and have boundaries that are difficult to discern (Travis 1965). The secretory activity of the epidermal Golgi is at a minimum (Chassard-Bouchaud and Hubert 1973), the endoplasmic reticulum is reduced (Green and Neff 1972) and the pore canals are structurally intact and may be partially filled with calcite crystals (Travis and Friberg 1963).

However, the epidermis is not completely inactive during intermolt. There is a peak in RNA synthesis during C_4 (Skinner 1966), chitin synthesis occurs at the same rate as in early premolt (Hornung and Stevenson 1971) and the reserve cells of the highly vacuolated and reduced connective tissue are prominent and are engaged in the storage of materials required for the next molt (Travis 1965).

Premolt Integument

In the classical molt-staging system, the onset of stage D_0 (premolt) is marked by epidermal secretion of enzymes, which causes solation of the membranous layer, freeing the epidermis from the cuticle and allowing it to retract. This retraction is called apolysis (Jenkin and Hinton 1966), the major landmark of stage D_0 (Drach and Tchernigovtzeff 1967). As noted earlier, there is reason to consider initial retraction to be the final stage of C_4 instead of D_0.

During early premolt, there is little change in the appearance of the epidermis, but the pore canals are severed (Green and Neff 1972), and there is an increase in cell height and structural complexity (Travis 1965). As premolt progresses from D_0 to D_4, the epithelial cells change from squamous to columnar and increase six-fold in height, reaching a maximum of 54 μm in Orconectes. However, not all cells follow this pattern. The reserve cells in the connective tissue of Orconectes decrease in size from 40 μm in stage C_4 to only 17 μm in stage D_0-D_1 (Travis 1965).

One of the first changes to occur in the epidermal cells during premolt is the proliferation of rough endoplasmic reticulum, indicative of increased protein synthesis. Golgi, which are inconspicuous during intermolt, also increase rapidly (Kummel et al. 1970). Mitotic activity is apparent in the epithelial cells during stages D_0, D_1' and D_1'', and protein and chitin synthesis and oxygen consumption peak during stage D_2 (Roer and Dillaman 1984).

The new epicuticle is secreted during early D_2, and exocuticle secretion begins in late D_2 (Travis 1965). Since these two layers are deposited before the molt (hence the designation 'preexuvial layers'), they are beneath the old cuticle and must remain sufficiently flexible to permit withdrawal at molt and increase in volume after molt. For these reasons, the organic matrix is deposited but calcification is postponed (Travis and Friberg 1963).

Degradation and resorption of the old cuticle continues from stage D_0 to late D_3. Although

portions of the old cuticle are resorbed as the new cuticle is deposited (cf Drach 1969; Green and Neff 1972), there is no evidence that material removed from the old cuticle is transferred to the new (Arsenault et al. 1984). The degree of cuticle resorption varies between species and even within the body of individuals (Roer and Dillaman 1984) but, in the areas such as the merus and the epimeral sutures, the endocuticle may be completely and the exocuticle partially resorbed. Remnants of the resorbed cuticle are thought to form the 'ecdysial membrane', a thin homogeneous, transparent layer that envelopes the animal during premolt (Aiken 1980; Travis 1965; Gnatzy and Romer 1984).

Resorption is accompanied by elevated hemolymph Ca^{++} and HCO_3^- concentrations and slight alkalosis (Roer and Dillaman 1984). An additional function of resorption is the conservation of organic and inorganic constituents other than calcium (Travis 1955). Although it has been questioned whether resorption can even occur once the pore canals have been disrupted (Green and Neff 1972), work on insects has shown that until the cuticle is made hydrophobic at ecdysis, the new cuticle is like a gut surface, unaffected by enzymes, but permeable to the resorbed products (Locke 1984).

Late in premolt, the epidermis and the newly secreted epicuticle and exocuticle undergo invagination and folding. This appears to be a mechanism for increasing the surface area of the new exoskeleton within the confines of the old, thereby enabling the animal to increase in size at molt (Aiken 1973; Arsenault et al. 1984; Vranckx and Durliat 1978).

Postmolt Integument

Dedifferentiation of the epidermal cells begins about 1 d after ecdysis (Green and Neff 1972). Within 2 d, the epidermal cells of Orconectes virilis have decreased from their maximal height of 54 µm to only 21 µm, a decline that continues until the minimum is reached in stage C_4 (Travis 1965).

After ecdysis, the exocuticle is tanned and the preexuvial layers are calcified. Tanning is by quinone formation from dihydroxyphenols under the action of a polyphenol oxidase transported to the cuticle from the epidermal cells (Roer and Dillaman 1984; Stevenson 1985). The preexuvial layers begin to calcify immediately after molt, during stage A or B. The first crystals of $CaCO_3$ are evident soon after molt and, in Astacus fluviatilis, calcium deposition reaches a peak 2 d postmolt (Welinder 1975a) while in O. virilis calcification does not begin until 2 d postmolt (Travis 1965). However, it is difficult to accurately determine onset of calcification and this may account for reported variability (Greenaway 1985).

Calcification of the epi- or exocuticle begins in the most distal regions and proceeds proximally, with mineral apparently reaching the outer portions of the cuticle via the pore canals (Travis and Friberg 1963). Calcium is concentrated in the distal portions of the epithelial cells and appears to be deposited in vertical rows that correspond to the position of the pore canals of the new cuticle (Travis 1963, 1965).

Endocuticular deposition marks the onset of either stage A_2 or stage B, depending on staging criteria used (see section on Stages of the Molt Cycle). Deposition of the endocuticle is completed by stage C_3, with each lamella being mineralized as it is laid down (Travis 1965). Mineral deposition continues throughout postmolt, ultimately pervading the walls and lumina of the pore canals as the cell processes recede and are replaced with mineral (Travis and Friberg 1963). The end of postmolt is marked by the end of net calcium deposition and the onset of membranous layer formation (Passano 1960).

CALCIUM METABOLISM

Rapid postmolt mobilization of stored calcium provides an endogenous source for cuticle calcification immediately after ecdysis. Stored calcium helps harden those parts of the body required for consumption of the cast shell and initial foraging (Greenaway 1985), and the calcium reserves of Procambarus clarkii are adequate for integumentary mineralization to stage C_1 (Huner et al. 1978). Chaisemartin (1964) suggested that stored calcium helps maintain ionic integrity of the hemolymph following water absorption at molt. Since calcium is not as readily available in freshwater habitats, calcium reserves are more important in fresh water than in marine species.

Exogenous calcium can be obtained from the diet or the medium and the relative importance of these two sources varies with the species. Generally, food (other than the exuvia) is thought to be only a minor contributor to the calcium requirements of aquatic species. The exuvia contains about 56% of the calcium present in the intermolt animal and is a ready source of calcium for postmolt mineralization.

Mechanisms exist in crayfish for the uptake and accumulation of calcium from dilute media via the gills. Low pH, absence of bicarbonate and low temperature reduce uptake. Calcium uptake is also dependent on food availability, and failure to feed postmolt animals causes a reduction in uptake (Greenaway 1985). In Austropotamboius, calcium uptake from the medium begins 15-30 min after molt and immediately increases to a maximum of 2 µmoles $Ca^{++} \cdot g^{-1} \cdot h^{-1}$. In this species, uptake decreases sharply between stages B_2 and C_1, although uptake continues throughout C_1-C_3 until equilibrium is reached at stage C_4 when calcification is complete (Greenaway 1985). Conversely, in O. virilis, lower calcium influx values were recorded and there was no sharp drop between stages B and C, perhaps reflecting the extremely low calcium level in the medium. This may indicate that calcium intake occurs during a larger portion of the molt cycle in soft water (Malley 1980).

Despite the importance of calcium, a significant amount is removed from the integument during premolt and is lost to the medium in soluble form. In A. pallipes, net loss increases in late stage D_0 and reaches a maximum of 0.8 µmoles $\cdot g^{-1} h^{-1}$ in stages D_3-D_4. Only 17% of the calcium present at intermolt remains after ecdysis, with a third of the loss occurring during premolt and the remainder at molt. Although there is no increase in ionized calcium in the hemolymph during premolt, there is an increase in complexed calcium (Greenaway 1985).

The carbonate required for mineralization may be obtained primarily from the cast shell and the diet, but other possible sources include HCO_3^-

from the medium and metabolic CO_2 (Roer and Dillaman 1984).

Little is known about the mechanisms for calcium and carbonate movement into and out of the mineralized structures in crustaceans (Roer and Dillaman 1984). Roer (1980) proposed a model for the bi-directional calcium transport required at different stages of the molt cycle, suggesting the epidermal cells are actively transporting calcium out of the cells on a continuing basis, and that it is the particular molt-related morphology of the cells that dictates the direction of net calcium movement. During postmolt, the squamous epidermal cells have large numbers of protoplasmic projections extending through the pore canals, and Roer suggested that such a large surface area results in net calcium movement to the exoskeleton. At premolt, changes in epidermal cell morphology reverse the surface ratio and thus the direction of net calcium movement.

Calcium peaks within the epithelial cells of shrimp occur during maximal cuticle resorption and deposition (Hubert and Chassard-Bouchaud 1978) and maximal in vitro resorption and deposition rates correspond to the molt stages at which maximal resorption and deposition occur in vivo (Roer and Dillaman 1984). The calcium transport mechanism involves both Ca-ATPase and Na-Ca exchange in conjunction with changes in epithelial cell shape and size (Roer 1980). Mineral must travel distances of 70 µm or more from the apical surfaces of the epidermal cells to harden the cuticle after ecdysis. The transport of calcium and, presumably, carbonate is thought to be effected by the epidermal cytoplasmic extensions of the pore canals since electron micrographs show mineral deposits in these areas (Travis and Friberg 1963).

Virtually nothing is known about the supply of carbonate to the cuticle, although carbonic anhydrase has been directly associated with calcification. This enzyme is localized within the epidermal cells and in the interprismatic regions of the exocuticle corresponding to the sites of calcification initiation. Although enzyme quantity peaks during postmolt calcification and innibition of the enzyme decreases rates of mineralization, the exact role of this enzyme remains obscure (Chockalingam 1971; Giraud 1981; Giraud-Guille 1984a).

Calcification of the new cuticle starts immediately after ecdysis and Digby (1984) postulated that mineral deposition results from precipitation mediated by electrical action between the quinone moieties and calcifying regions in response to the generation of pH gradients. An alternative offered by Giraud-Guille (1984a) is that the presence of cation-binding glycoproteins at the sites of calcification initiation induce the precipitation phase. Roer and Dillaman (1984) suggested that a nucleating agent is secreted within the matrix of the cuticle (or an inhibitor of nucleation is removed), enabling calcification to proceed. They arrived at this conclusion after observing that in vitro mineralization would occur within the postmolt cuticle but not the premolt cuticle of crabs. In this context, it is interesting that premolt crabs prematurely removed from their shells proved unable to calcify their new cuticles (Paul and Sharpe 1916).

Calcification begins in the external laminae of the exocuticle and along the interprismatic septa (prints in the exocuticle left by the margins of the epidermal cells). The rapid onset of postmolt calcification in the distal laminae of the exocuticle coincides with maximum calcium uptake and carbonic anhydrase activity and the presence of calcium-binding glycoproteins (Giraud-Guille 1984a).

Calcification of the new cuticle ceases at the end of stage C_3, at the time the non-calcified membranous layer starts to form. Drawing on results from carapace repair studies, Roer and Dillaman (1984) suggested that it may be secretion of the membranous layer that curtails further crystal growth in normal cuticle deposition.

Gastroliths

The paired gastrolith discs are differentiated epithelium on the lateral walls of the cardiac stomach. Their function is to collect calcium from the hemolymph and secrete it in the form of calcium carbonate concretions called gastroliths. At ecdysis, the gastroliths are deposited in the lumen of the stomach where they are converted to ionic calcium for use in postmolt mineralization. However, calcium stored in the gastroliths only amounts to about 10% of the intermolt calcium and only 4-7% of the animal's requirement in the next cycle (Greenaway 1985).

The gastrolith discs consist of cuticular lining, epidermis and basement membrane. Unlike the normal stomach epidermis, which consists of cuboidal or low columnar cells, the epidermis of the gastrolith disc has but a single layer of columnar cells. It also lacks the membranous layer found in the cuticular lining of the stomach, its place being taken by the gastrolith crystals (Suko 1968; Travis 1960).

In stage D_0, the gastrolith disc undergoes rapid change. The epidermal cells increase in height and become branched and attenuated and intercellular spaces develop. The apices of the attenuated cells are in contact with the developing gastrolith matrix and are responsible for its formation (Travis 1960). As premolt progresses, the gastroliths increase rapidly in thickness. In Orconectes virilis, they can increase from 300 µm in D_0 to as much as 3-4 mm in stage D_4 (Travis 1960), and contain about 20% of the calcium resorbed from the cuticle. Of the calcium remaining after ecdysis, 60% is found in the gastroliths (Greenaway 1985).

Untrastructural studies on the gastrolith epidermis of Procambarus clarkii showed cells that display properties of a transport epithelium with elaborate microvilli, and demonstrated calcium accumulation in the epithelial mitochondria. An active role of mitochondria in calcium translocation has been suggested, with the mitochondria effecting the transepithelial movement of calcium during elaboration of the gastroliths. Data also suggest that Ca^{++}-ATPase plays an important role in calcium transport in the gastrolith (Mizuhira and Ueno 1983; Ueno 1980; Ueno and Mizuhira 1984).

Gastrolith deposition is completed by stage D_4 and the epidermis then retracts from the crystals and grows rapidly. New gastrolith

epicuticle is deposited just before ecdysis (Travis 1960; Suko 1968) and, after ecdysis, this is the only cuticular layer present above the folded epidermis. The epidermal cells then increase in height and exocuticle is deposited (stages A and B). Unlike the exocuticle of the general integument, gastrolith exocuticle is uncalcified and lacks pore canals and tegumental glands (Travis 1960).

Endocuticle deposition begins on the gastrolith disc in late stage B and is completed by stage C_4. It is the thickest of the three layers (15-25 μm) but is non-laminar. Like the gastrolith exocuticle, it lacks pore canals and tegumental glands (Travis 1965).

GROWTH

In crustaceans, the real growth processes of cell proliferation and protein synthesis are periodic and out of phase with the obvious increases in body length and volume that are normally used to measure growth. Protein synthesis and cellular proliferation occur primarily during the intervals between molts, whereas increases in length and volume occur immediately after ecdysis, once the real growth processes have ceased.

Size increase at molt is extremely variable in crustaceans (Hartnoll 1982, for review) and this, combined with wide variations in the frequency of molting, complicates the analysis of crustacean growth and the processes that control it. Furthermore, molt frequency and size increase at molt are often affected in different ways by changes in the environment. As a result, crayfish of similar age may vary widely in size, and a size-class may encompass a variety of ages, particularly at the larger end of the size range (Brewis and Bowler 1982).

CHARACTERISTICS OF CRAYFISH GROWTH

When the premolt length of a crayfish is plotted against the postmolt length of that same animal for a succession of molts, the straight line of a Hiatt growth diagram is produced (Fig. 6). The same can be done with premolt and postmolt weight. Hiatt growth diagrams have been constructed for Cambaroides, Pacifastacus, Paranephrops, Austropotamobius and Orconectes (Brown and Bowler 1979; Flint 1975; Hopkins 1967; Jones 1981; Kurata 1962; Mason 1974; McGriff 1983; Price and Payne 1984).

When growth of an animal from hatching to maturity is plotted in this way, the changes from one growth phase to another may show up as inflections in the line. For example, growth characteristics may change from the larval to the juvenile phase and again when the animal becomes sexually mature. Each of these changes may produce an inflection in the line.

Kurata (1962) characterized three types of growth from the slope of the line in a Hiatt growth diagram: progressive geometric, in which linear increment increases with animal size; retrogressive geometric, in which linear increment decreases with size; and arithmetic, in which linear increment remains constant and is independent of size.

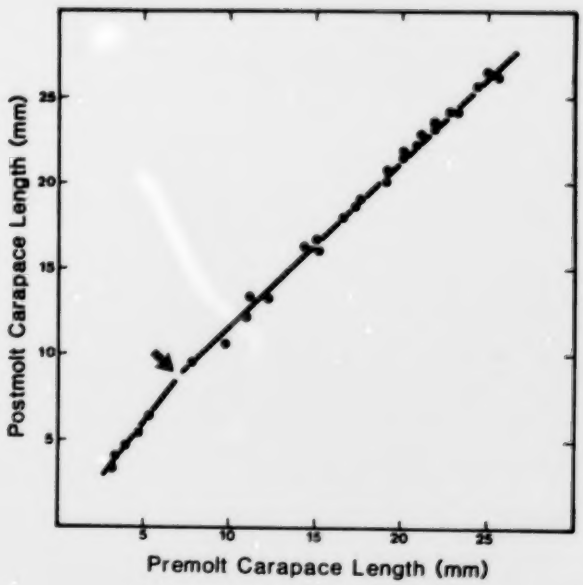

Fig. 6. Hiatt growth diagram of pre- and postmolt carapace lengths for Cambaroides japonicus. Arrow indicates inflection point that corresponds to approximately stage 6, and may indicate a change in growth pattern following metamorphosis (from Kurata 1962).

In the first instars of Cambaroides and Paranephrops, growth is progressive geometric, but in the juvenile and mature phases, growth becomes arithmetic (Jones 1981; Kurata 1962). In Procambarus hayi, on the other hand, growth remains progressive geometric through the juvenile and adult stages (Payne 1972). In Austropotamobius, Pacifastacus and Orconectes, growth is progressive geometric from the juvenile to the young adult stages but then declines, producing an inverse relationship between body size and relative growth increment (Brewis and Bowler 1982; France 1985; Mason 1975). These examples illustrate the considerable interspecific differences in the growth characteristics of crayfish.

There is also pronounced variation in the growth rate of individuals within a species. The growth rate of an individual animal results from the interaction of two components: frequency of molting and size increase at molt. As previously noted, these two can be differentially affected by the physiological state of the animal and the environmental conditions it encounters.

Thus, it is possible for individuals within a year-class to grow at widely differing rates and, since the cumulative effects will be more pronounced when the population is growing rapidly, the greatest variation within a year-class occurs in the first year (Flint 1975; Hopkins 1967). Carapace lengths of O. propinquus, for example, can vary from 12-27 mm by the end of the first growing season (Van Deventer 1937).

In general, the growth rate of crayfish is slowed by advancing age and reproductive function, and molt frequency and linear increment both tend to

decrease as the animal ages (Avault and Huner 1985; Brewis and Bowler 1982; Flint 1975; Jones 1981; Kurata 1962). Superimposed on this are the demands of reproductive growth, which tend to depress the linear increment in females of some species (Brewis and Bowler 1982; Hopkins 1967), and the effects of the environment, which may retard either or both the molt frequency and the increment at molt.

Allometric Growth

The changes in body form that occur in a growing crustacean are referred to as relative or allometric growth. Briefly, allometry is the relationship of two changing dimensions, one (such as carapace length) is termed the reference dimension or independent variable (X), and the other (leg length, cheliped depth, abdomen width) is the dependent variable (Y). Allometric growth is important because changes in the level of allometry often reflect important physiological events in the life history of the animal (eg. onset of sexual maturity).

Allometric growth is represented by the formula $Y = aX^b$, where a is the Y intercept and b the allometric growth constant. In practice, it is usually transformed to the logarithmic form:

$$\log Y = \log a + b \log X$$

to yield a straight line when $\log Y$ is plotted against $\log X$. If the slope, b, is greater or less than 1, the allometric relationship is said to be either positive or negative. If $b = 1$, the relationship is isometric.

Positive allometry exists for the chelipeds of mature male crayfish and the abdomen width of mature female crayfish when compared to carapace length (Fig. 7). Conversely, female and immature male chelipeds tend to grow isometrically (Mason 1974; Price and Payne 1984; Romaire et al. 1977, but see Stein et al. 1977). In some species, both the length and the width of chelae show positive allometry (Price and Payne 1984), but in others positive allometry may exist in only one dimension (Lindqvist and Lahti 1983).

ENVIRONMENTAL AND OTHER INFLUENCES

Environmental signals mediated through the central nervous system regulate the physiological and morphological changes associated with the molt cycle. Growth is modified by external stimuli and the responses of crayfish to these stimuli may be highly variable, even among closely related species. Some environmental factors affect molt frequency while others alter the size increase at molt. Food, temperature and population density have been identified as the most important environmental factors influencing crayfish growth rates, but there are few endogenous or exogenous factors that do not affect molting and growth in some way. Although temperature is usually considered the most influential, growth can vary widely between habitats even when temperatures are similar (Flint and Goldman 1977; France 1985). Adverse conditions such as crowding, food shortage or unfavorable temperatures can delay molting by 6 mo or more (Huner and Avault 1977). In addition, molt stage plays a role: crayfish are more sensitive to adverse environmental conditions during the late premolt and early postmolt stages (Avault and Huner 1985).

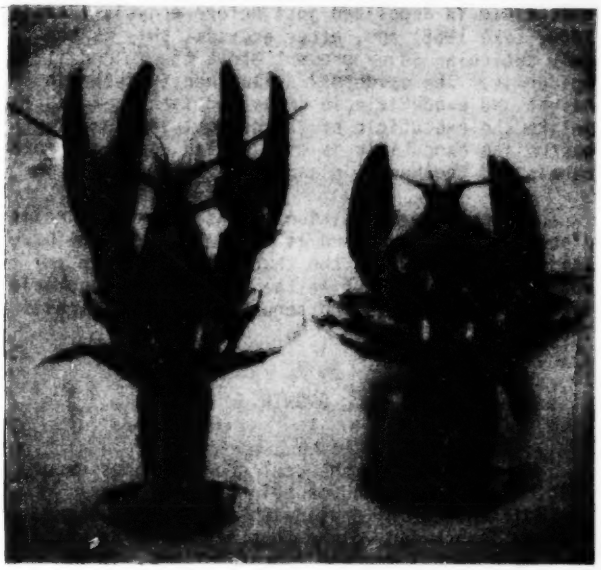

Fig. 7. Positive allometric growth of male chelipeds and female abdomen width in Orconectes virilis. The form I male (left) and the mature female shown here have identical carapace lengths (51 mm).

Temperature and Latitude

Higher latitudes generally have lower mean annual temperatures and, since crayfish growth rates tend to increase with temperature, the fastest growth for a species with a north-south distribution will usually be toward the southern end of the range.

However, the relationships between latitude, temperature and growth can be complex. In northern areas, the sub-freezing conditions of winter can suspend growth of a crayfish such as Orconectes virilis for several months of the year (Fig. 8). But in southern areas the heat of summer can be just as effective in suspending growth, driving crayfish such as Procambarus deep into the mud. Thus, crayfish in the southern United States tend to grow from autumn through winter and into spring, but not during the heat of summer, whereas those in the northern United States grow from spring through summer and into autumn, but not during the cold of winter.

Optimum temperatures for growth of Procambarus range from 20-25°C, but growth is not drastically reduced until the temperature falls below 13°C or rises to 32°C (27°C in the case of P. a. acutus - Lutz 1983). For many crayfish species, 10°C is the lower limit for growth, and year-to-year variations in days above 10°C can cause annual differences of 8-15% in the growth rate of O. virilis (France 1985).

Fig. 8. Conditions experienced by Orconectes virilis in a stream habitat at the northern extremity of the range (Alberta, Canada). These crayfish spend 5-6 mo huddled beneath rocks at near-freezing temperatures. Those that are trapped in nearshore areas that freeze to the bottom will perish (foreground).

The most rapid growth of P. clarkii occurs in the late autumn and early spring and, under ideal conditions, juveniles may molt at 6-10 d intervals (Avault and Huner 1985). If water temperature remains high, market size can be attained in 60-90 d (Huner and Barr 1984). At higher latitudes, this species requires 90-105 d just to reach the smaller bait size (Huner 1983). O. virilis, on the other hand, may require 3 yr to reach market size in the northeastern United States (Nolfi 1980), and northern European Astacus often require more than 4 yr (Tcherkashina 1977).

Photoperiod

Temperature has a general influence on metabolic processes, but the effects of light are more specific and can be expressed either directly or indirectly. Direct effects, such as photoperiodism, operate through the central nervous system to control molting, but indirect effects, such as those of light intensity, often influence growth through a secondary mechanism such as behavior or feeding activity.

Photoperiod regulates molting in some crayfish species. Long daylengths generally favor molting (Armitage et al. 1973; Stephens 1955), but aperiodic conditions, such as constant light or constant dark, have mixed effects. Constant darkness appears to stimulate molting in young Cambaroides but inhibit it in Orconectes, and constant light often inhibits somatic growth, thereby increasing intermolt times (Kurata 1962; Mobberly 1963; Stephens 1955).

The effect of a given photoperiod will vary according to the animal's environmental history (Aiken 1969) but, if crayfish are held in temperatures above the physiological minimum, all will eventually molt, irrespective of photoperiod. Crayfish held at favorable temperatures in long-day photoperiod tend to molt continuously, even during seasons of the year when molting would not normally occur, but those held on normal and short-day photoperiods will not molt during seasons when the normal daylength is decreasing (Rice and Armitage 1974). These observations are consistent with the role of photoperiod in the seasonal timing of molting.

Seasonality

Molting in most species occurs primarily in seasons of optimum temperature, and at the extremes of the range the optimum seasons may be very short. This is especially true in northern species such as O. virilis (Fig. 9) and Austropotamobius pallipes.

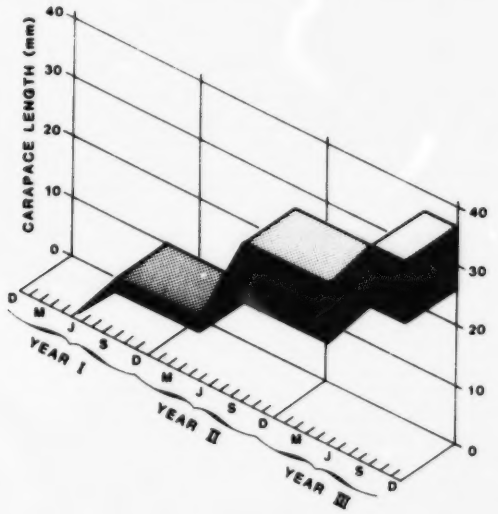

Fig. 9. Growth pattern of a year-class of stream dwelling Orconectes virilis during 3 yr at the northern extremity of its range (Alberta, Canada). A relatively brief period of growth during summer is followed by a long interval of no growth during autumn and winter.

Species in lower temperate latitudes are not as constrained by temperature and therefore molt throughout a greater portion of the year.

Crayfish utilize environmental cues to synchronize molting to appropriate seasons, thereby ensuring completion of the molt before environmental conditions become unfavorable. In some cases, the regulatory parameters are easily identified (Aiken 1969) but, in others, both the regulation and response are more subtle (Jegla 1966). For O. virilis in northern latitudes, temperatures are favorable for molting in only 2-3 mo during the summer (Fig. 9). It is therefore important that the animal be ready to molt when the temperature rises in late spring, and to complete their growth before

the temperature drops in late summer. The opposite situation may exist for summer populations, where summer heat and low water levels suppress molting and force the animals to burrow for survival. In either case, the molting cycles tend to be coordinated with the seasons and the animals will be predominantly in molt stage C_4 or D_0 during the unfavorable period (Huner and Avault 1976a).

Hydrological Cycles

The life history of P. clarkii and P. a. acutus is closely linked to annual hydrological cycles (Avault and Huner 1985), and local success of these species is largely dependent upon temperature and the timing of hydrological events (Sommer 1984). Similar factors may be involved in the life cycle of cave crayfish (Jegla 1966).

Fastest rates of growth occur in P. clarkii when predictable low water periods alternate with a wet season, as occurs in Louisiana culture ponds (Sommer 1984). These cycles of draining and flooding enhance growth by synchronizing crayfish reproduction, eliminating fish and insect predators and generating vegetation for food and cover (Huner and Barr 1984).

Density

Population density is a key factor in determining growth rate of crayfish (Chien and Avault 1983; Flint and Goldman 1977; Hopkins 1966; Mills and McCloud 1983; Momot et al. 1978; Morrissy 1979). Both field and laboratory studies have revealed an inverse relationship between population density and crayfish size (Abrahamsson 1966; Abrahamsson and Goldman 1970; Flint and Goldman 1977; Goyert 1978; Hopkins 1966; Momot et al. 1978).

In Cherax tenuimanus, density and growth rate are inversely related, and time to market size varies from 14 mo to more than 3 yr, depending on density (Morrissy 1979). Chien and Avault (1983) suggested that crowding may impair growth irrespective of food abundance because of a 'space factor' (Hile 1940), a phenomenon noted also in lobster culture (Van Olst et al. 1980, for review). Goyert and Avault (1979) found that small containers produced the slowest growth rate and the earliest cessation of growth in P. clarkii.

Stunting in P. clarkii due to overcrowding and shortage of forage is a major problem in pond culture, where the majority of stunted Procambarus often fail to reach the minimum legal size of 75 mm TL (Huner and Barr 1984). Interestingly enough, there is no apparent relationship between population density and the size range of crayfish in ponds (Momot and Romaire 1981).

Habitat and Substrate

There can be considerable variation in growth of crayfish in different habitats (Flint and Goldman 1977; Shimizu and Goldman 1983), but some species can apparently adapt to different habitats without a significant change in growth pattern (Flint 1975).

Bottom cover and shelter are important to the growth and survival of crayfish (Flint and Goldman 1977; Huner 1978; Mason 1979; Westman 1973). Shelters will increase the survival of P. clarkii in high density growout systems (Goyert 1978) and, in ponds, P. clarkii use vegetation to segregate themselves (Huner 1978). Changes in the amount and type of vegetation can affect the food chain and the availability of cover. After ponds are flooded in the autumn, there is an abundance of vegetation, but this can be rapidly reduced by grazing crayfish. The reduction in vegetation suppresses the growth of crayfish that remain in the pond (Avault and Huner 1985).

Mason (1979) found that shelters enhanced the survival of crayfish in tanks but had no effect on growth rate. Goyert (1978) obtained similar results at stocking densities of 20-25/m^2, but found that both growth and survival were depressed when the stocking density was increased to 100/m^2.

During years of low water levels, Procambarus may hatch and remain in the burrows, and the young crayfish may suffer from overcrowding and lack of food. Growth of the young is greatly enhanced if open water is available (Bardach et al. 1972). It has also been suggested that juvenile crayfish in flowing waters grow less at molt than those in pools or lakes due to the energy required to maintain their position in flowing water (Flint 1975; see also Hopkins 1967).

Water Quality

There is surprisingly little information on the effects of trace elements and pollutants on crayfish molting. Elevated levels of aluminum appear to decrease calcium uptake (Malley and Chang 1985), and studies on other crustaceans suggest that molting and growth of crayfish would be adversely affected by cadmium, zinc, methylmercury, aromatic hydrocarbons, PCB's, ammonia, chlorophenols, dithiocarbamates and a variety of pesticides (Weis 1985 and Reddy et al. 1985 for references). Some contaminants decrease growth at molt and reduce molting incidence, while others interfere with cuticle formation or ecdysis, causing death at molt.

Adult intermolt crayfish are reasonably tolerant of acidified waters, but juvenile and postmolt animals are sensitive (Morgan and McMahon 1982). Calcium uptake after ecdysis is impaired when the pH is below 5.75, and is inhibited when it is below pH 4.0 (Malley 1980). Sublethal acidic conditions appear to cause acidosis which leads to cuticle breakdown, reduced calcification and unusually thin shells (Morgan and McMahon 1982). There is also evidence that reduced pH increases susceptibility to Thelohania infection (France and Graham 1985).

Growth and survival generally improve with increasing water hardness. A total hardness of 100-150 ppt is recommended for pond culture (de la Bretonne et al. 1970; de la Bretonne and Avault 1971), but acceptable crayfish production has been obtained in ponds where the water hardness was less than 15 ppt (Huner 1978). In such situations, the crayfish may be obtaining the required calcium from their diet.

Low oxygen levels often cause high mortality in crayfish ponds (Avault et al. 1975), and small animals and those in late premolt and ecdysis are particularly sensitive (Melancon and Avault 1977). Growth rates of pond crayfish improve with increasing levels of dissolved oxygen (Chien and Avault 1983) and, in sublethal oxygen conditions, crayfish feeding and growth at molt are reduced (LaCaze 1976; Morrissy 1979).

Many crayfish species are tolerant of brackish conditions and can survive for up to 2 mo in salinities of 15-20 ppt (Avault and Huner 1985). Growth rate generally decreases in proportion to increasing salinity (Sharfstein and Chafin 1979), but Procambarus will grow fairly well even at salinities as high as 6-10 ppt (Bardach et al. 1972).

Maturity and Reproduction

Somatic and reproductive growth processes compete for a limited energy reserve in decapods. This is often reflected in reduced somatic growth, especially among females in which significant energy is diverted into ovarian growth. For this reason, the growth rate of mature males is often faster than that of mature females. In Astacus, the linear increase of mature males is more than twice that of the females of the species (Abrahamsson 1971, 1972) but, in Austropotamobius, only the post-reproductive molt of females is adversely affected (Brewis and Bowler 1982). Austropotamobius is interesting in that the growth differential between the sexes is significant only in the smallest reproductive sizes. In Orconectes propinguus, O. n. chaenodactylus and Pacifastacus, both sexes grow at the same rate (Creaser 1934; Flint 1975; McGriff 1983; Price and Payne 1984; but cf. Abrahamsson 1971).

In addition to these indirect effects of reproduction on molting and growth, direct inhibition can occur. For example, the presence of eggs on female pleopods can suppress the spring molt (Scudamore 1948; Tack 1941) such that adult females of most crayfish species will molt only once a year while males are molting twice (Aiken 1969; France 1985; Pratten 1980; Tack 1941; Van Deventer 1937). If the eggs are removed from the pleopods before the normal hatching time, the ensuing molt will be accelerated.

Nutrition

Molt frequency appears to be limited by the rate of tissue growth following ecdysis (Adelung 1971). This suggests that nutrition influences growth primarily by regulating the frequency of molting, and there is empirical evidence to support this. In Cambaroides, reduced rations cause extended intermolt times (Kurata 1962) but, if the animal is already in premolt, starvation will suppress gastrolith growth and prolong premolt (Rao et al. 1977). Starved Faxonella tend to delay initiation of premolt until feeding resumes and, if food is withheld long enough, Faxonella will die without entering premolt. In Homarus the relationship that has been noted between serum protein level and intermolt time suggests a direct effect of nutrition on molt (Aiken 1980).

Procambarus reared intensively have been found to grow larger on a diet that contains vegetation (Huner 1984), which probably explains why crayfish production in a pond increases in proportion to the amount of rice and other vegetation produced in the pond. Lack of vegetation in a pond can increase aggressive interaction and competition between animals (Cange et al. 1982) and cause stunting at submarket sizes (Avault and Huner 1985).

Green vegetation contains carotenoids, and carotenoids give the crayfish integument its normal pigmentation. In the absence of carotenoids the integument becomes grey or pale blue and the hepatopancreas is small and off-color, deficiencies that can be corrected by supplementing the diet with green plant material. However, the physiological importance of dietary carotenoids is not well understood, and no differences in growth rate or reproductive success have been noted in cage-reared crayfish fed diets apparently deficient in carotenoids (Huner and Meyers 1979).

Food quality is even more important in intensive culture where all trace elements and essential vitamins must be supplied to the animal in a prepared ration, and when growth is manipulated by eyestalk ablation and other techniques that have a direct effect on metabolism (Chang and O'Connor 1983; Smith 1940).

Parasites and Disease

Thelohania sporozoans and the fungus Aphanomyces astaci have had a serious impact on the native European astacids, but no major diseases affect cultured species of North American crayfish. External infections by chitinivorous bacteria and protozoan epibionts are controlled by the frequency with which crayfish molt (Avault and Huner 1985).

Infestation by the parasite Thelohania contejeani reduces the growth increment of adult male and non-producing female Austropotamobius pallipes. The small increment typical of the post-reproductive molt of females is not affected by the parasite, and Brewis and Bowler (1982) suggested that the energy demand on reproduction depresses growth to a point beyond which it cannot be further reduced by Thelohania parasitism.

ENDOCRINE MECHANISMS

The current paradigm of decapod molt regulation is as follows: a molt-inhibiting hormone (MIH) from the X organ-sinus gland complex of the eyestalk suppresses the biosynthetic activity of the molting glands (the so-called Y organs). Once released from this inhibition, the Y organs synthesize and secrete ecdysone, and this in turn initiates a suite of biochemical and physiological changes that culminate in ecdysis. Environmental factors that influence molting are assumed to act through the central nervous system (CNS) to control the synthesis and release of MIH. Thus, molting in crustaceans is often presented as a relatively straightforward system involving two antagonistic hormones, one a molt-inhibiting neuropeptide, the other a molt-promoting steroid. In reality, crustacean molting physiology is a profoundly complex system about which much is known but little is clearly understood. A few of these complexities and uncertainties are explored in the following sections.

MOLT INHIBITION

Molting in decapods is thought to be regulated primarily by molt inhibiting hormone (MIH), a protein or peptide produced and secreted by the neurosecretory cells in the X organ-sinus gland complex in the eyestalk (Fig. 10). The nature and mode of action of this putative hormone are still poorly defined, and attempts to purify it have been hampered by insufficient material and lack of a sensitive bioassay. Recently, Webster and Keller (1986) isolated a 61-residue peptide with a minimum molecular mass of 7200 Da from the sinus glands of

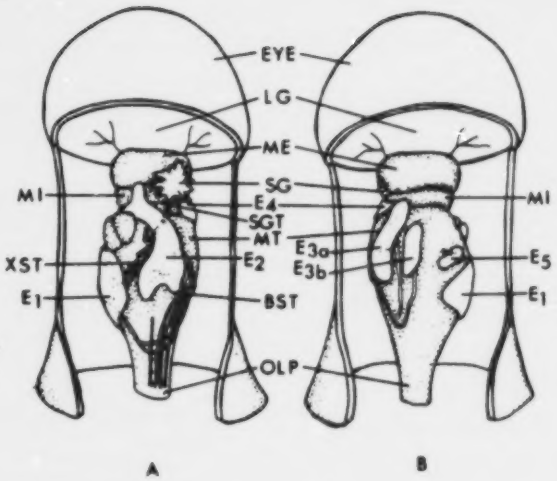

Fig. 10. Neuroendocrine structures in the right eyestalk of Orconectes virilis. Dorsal (A) and ventral (B) views. E1 through E5 are groups of neurosecretory cells that comprise the X organ. SG, sinus gland; SGT, sinus gland tract; XST, X organ-sinus gland tract; BST, brain-sinus gland tract; LG, lamina ganglionaris; ME, medulla externa; MI, medulla interna; MT, medulla terminalis; OLP, optic lobe peduncle (from Bliss et al. 1954).

Carcinus. This putative MIH proved effective at repressing ecdysteroid synthesis in vitro, and is active at concentrations of less than 250 pmol/L. In Homarus, Change et al. (1987) have isolated two closely related peptides with molt inhibiting activity that have a molecular weight of approximately 8700.

Eyestalk ablation causes a rapid increase in hemolymph ecdysteroid (Jegla et al. 1983; Keller and O'Connor 1982), and implanted lobster sinus glands delay molt in destalked crayfish (Couch et al. 1976). In addition, ecdysteroid secretion by crayfish Y organs is enhanced by eyestalk ablation (Keller and Schmid 1979), but is suppressed in crayfish (and crabs) when the glands are cultured in media conditioned with sinus glands, eyestalk extracts or cyclic AMP (Gersch et al. 1980a, b; Jegla et al. 1984; Mattson and Spaziani 1985a, b; Soumoff and O'Connor 1982; but see Gersch and Bohm 1982).

Although there seems little question that MIH inhibits Y organ activity, there is other evidence that MIH may modify the response of target tissues to molting hormone. It may be that regulation of hormone production and modification of target tissue response are but two of a multiplicity of MIH effects.

In crabs, Y organ inhibition by eyestalk tissue is reversible, dose-dependent and reproducibly linear within the range of 0.1-4.0 eyestalk equivalents (Mattson and Spaziani 1985a), and it appears that sinus glands can prevent activation of the Y organ and suppress previously activated glands (Keller and O'Connor 1982). Other studies show that Y organs from destalked crabs take up less cholesterol than controls when cultured with eyestalk extract (Watson and Spaziani 1985), and that the neurotransmitter 5-hydroxytryptamine mediates MIH release in a reversible dose-dependent manner, apparently through excitatory input to MIH-containing neurosecretory cells (Mattson and Spaziani 1985c).

These experiments all support the concept that MIH regulates molting by influencing ecdysteroid production by the Y organs. However, there is considerable evidence that MIH can modify target tissue response to circulating ecdysteroids. Aiken (1969) reached this conclusion when the photoperiodically regulated responses of northern Orconectes virilis proved difficult to explain with the classical hypothesis. Additional support came from studies by Warner and Stevenson (1972), Buchholz and Adelung (1979) and Lowe et al. (1968). Direct evidence that the relative titers of MH and MIH influence tissue response was provided by Freeman and Costlow (1979), who found that shrimp eyestalks (eg. MIH) interfered with ecdysteroid-induced apolysis in barnacle mantle, but that inhibition could be overcome by higher doses of 20-OH-ecdysone (see also Freeman 1980; Freeman and Costlow 1984).

Finally, the traditional concept of decapod MIH-MH relationships has been further stressed by suggestions that decapods are able to regulate their ecdysteroid levels in a normal manner even when there is no eyestalk tissue (Chang 1985), and that molting ecdysteroids may originate from sources other than the Y organs (Chang et al. 1976; De Leersnyder et al. 1981; Hopkins 1985).

MOLT INDUCTION

In the classical view, molt induction has occurred when a decapod in molt stage C_4 enters D_0 and begins the complex physiological and biochemical processes that culminate in ecdysis. However, in some decapods, including Procambarus clarkii and the American lobster, the protracted pause between one molt and the next (anecdysis) occurs not at C_4, but in stage D_0. Lobsters and crayfish may remain in D_0 indefinitely but, once the animal has entered stage D_1, additional developmental plateaus are rare (Aiken 1973; Huner and Avault 1976a). Stage D_0 includes a number of events that are characteristic of premolt: apolysis, limb regeneration and onset of setal and gastrolith development. However, the onset and rate at which these occur during D_0 is extremely variable and it is only when the animal reaches stage D_1 that premolt events proceed irreversibly at a rate that is dependent upon animal size and temperature. Because of this, 'molt induction' more logically refers to the transition from D_0-D_1 than from C_4-D_0 (Aiken 1973, 1980).

Although an eyestalk molt accelerating factor has often been suggested, the weight of current opinion favors passive molt induction in crustaceans, premolt being induced and sustained by the absence (or relative insignificance) of MIH. In insects, on the other hand, molt induction is thought to be an active process, molt gland activity being stimulated by a neurosecretory hormone from the corpora cardiaca. Irrespective of the manner of molt gland regulation, it appears that the molting process in both insects and crustaceans is initiated by ecdysone secreted by the molt gland.

Molting Gland

The molting gland, or Y organ, is an endocrine structure of ectodermal origin located bilaterally beneath the epidermis of the anteriolateral carapace (Fig. 11). There is strong evidence that the physiological changes of premolt are induced and regulated by secretions from these glands.

The Y organs of lobster and crayfish are an integral part of the hypodermis and are, therefore, less conspicuous than the Y organs of crabs, which are detached from the hypodermis. This caused the Y organs and the mandibular organs of astacurans to be confused in some early studies, a fact first reported in 1972 by Sochasky et al. (for reviews, see Aiken 1980; Kleinholz and Keller 1979; Spindler et al. 1980).

Once the Y organs of Procambarus and Orconectes were correctly identified (Aoto et al. 1974; Burghause 1975), ablation studies revealed that Y organ removal in early stage D inhibited gastrolith growth and prevented molting in a significant percentage of crayfish (Burghause 1975; Keller and Willig 1976). Crayfish responses were therefore similar to those of crabs in which Y organ removal prior to stage D_2 would block the ensuring molt, but removal after the onset of stage D_2 (maximal ecdysteroid titer) was ineffective (Echalier 1954).

In crayfish (at least in Orconectes), ecdysone is found mainly within the mitochondria of the molt gland. In insects, on the other hand, ecdysone occurs predominantly in the smooth endoplasmic reticulum. This suggests substantial differences in the biosynthesis of ecdysone in the two taxa (Birkenbeil and Eckert 1983). In addition, the molting gland is only one of several known sources of molting hormone in insects (for review, see Gnatzy and Romer 1984), but the Y organ is generally thought to be the principal source in decapods. Ecdysteroids have been identified in the ovaries of Carcinus (Lachaise et al. 1981), and the eyestalks of Uca (Hopkins 1985), but the ovarian ecdysteroids appear to be contained by that organ, and the eyestalk ecdysteroids do not, as yet, have a demonstrated function.

Two other previously suspect tissues have been dismissed as producers of molting hormones: the mandibular organs (see below), and the 'cephalic glands'. These latter, once thought to release ecdysteroids in vitro (Gersch et al. 1979), are no longer considered to be endocrine tissue or to be involved in molt regulation (Böhm and Gersch 1983; Jegla et al. 1983).

Molting Hormones

'Molting hormone' (MH) is a general term for endocrine factors that induce premolt and the physiological and biochemical changes that lead to molt. The major ecdysteroids in decapods are ecdysone, 20-OH-ecdysone and ponasterone A. Most studies have found 20-OH-ecdysone to be the predominant hormone, both in whole animal extracts and in the hemolymph (Chang 1985, for references). However, in Cancer antennarius, both ecdysone and 20-OH-ecdysone are found in comparable amounts in the hemolymph (Vensel et al. 1984), and recent studies have indicated that ponasterone A may also be important (Lachaise and Lafont 1984; McCarthy 1982).

Y organs may secrete ecdysone alone (Chang 1985), in combination with small quantities of 20-OH-ecdysone (Keller and Schmid 1979), or with considerable quantities of an unknown but less polar ecdysteroid that has five times the activity of ecdysone (Watson and Spaziani 1985). In crabs, Y organs secrete both ecdysone and 25-deoxyecdysone, which after hydroxylation in peripheral tissues yield 20-OH-ecdysone and ponasterone A (Lachaise et al. 1986). Cholesterol appears to be the ecdysone precursor (Vensel et al. 1984; Willig and Keller 1976) and, since crustaceans do not synthesize cholesterol de novo, it must be taken up from the hemolymph. In early premolt, the Y organs rapidly take up and convert this sterol precursor and secrete ecdysone (Vensel et al. 1984).

In vertebrates, steroid hormones are bound to carrier proteins in the blood for transport to target tissues. Here the steroid enters the cell by passive or facilitated diffusion in combination with an energy dependent process. This is the uptake system, a mechanism with relatively low specificity. The steroid is then bound by a specific macromolecular receptor in the cytoplasm and the resulting receptor complex is activated and translocated to the nucleus, where it attaches to acceptor sites on the chromatin and acts as an intracellular gene regulator by altering specific RNA and protein synthesis (Londershausen and Spindler 1985).

Recent work indicates crayfish steroids are handled in much the same way, except there appear to

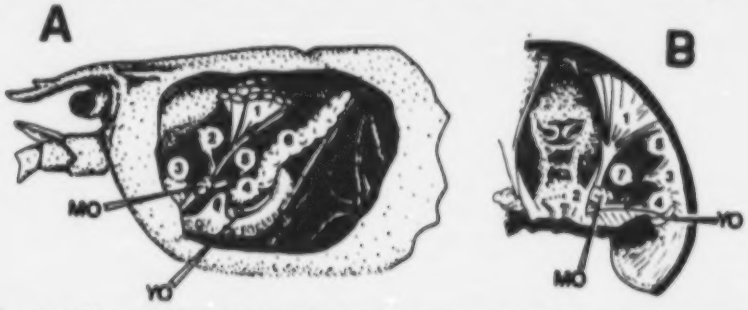

Fig. 11. Location of the molting gland or Y organ (YO) and mandibular organ (MO) in crayfish, as viewed from the lateral (A) and posterior (B) aspects. Muscles and tendons identified: (1) posterior mandibular adductor, (2) anterior dorsoventral, (3) lateral mandibular adductor, (4) posterior dorsoventral, (5) maxillulary abductor coxopoditis, (6) epimeral, and (7) major mandibular abductor.

be no binding proteins in the hemolymph (Londershausen and Spindler 1985). Ecdysteroid receptors (binding proteins) have been demonstrated in the cytosol and nuclei of several tissues in Orconectes and Astacus (reviewed in Skinner 1985b; Londershausen and Spindler 1985; Spindler et al. 1984). These receptors have the highest affinity for ponasterone A, followed by 20-OH-ecdysone and then by ecdysone, and these affinities are generally higher than are found in insects, perhaps because of the somewhat lower circulating ecdysteroid titers of decapod crustaceans.

The high affinity for ponasterone A is interesting since the molting gland produces ecdysone which is hydroxylated to 20-OH-ecdysone, and this latter is generally considered to be the predominant molting hormone. What then is the role of ponasterone A, and why do the ecdysteroid receptors show such high affinity for it? Unfortunately, the origin and function of ponasterone A is not yet known but it is not a metabolite of ecdysone. It appears to be important in premolt development, and it increases rapidly during stages D_1-D_2 to a level several times that of 20-OH-ecdysone, and then decreases rapidly during stages D_3 and D_4 (McCarthy 1982). Lachaise and Lafont (1984), noting that ponasterone A is a major ecdysteroid of crab hemolymph, suggested that it, like ecdysone, could be a precursor of 20-OH-ecdysone. Such a mechanism might explain some of the puzzling and occasionally contradictory results that have been obtained with eyestalk and Y organ ablations.

As the foregoing discussion illustrates, our understanding of the metabolism and control of molting hormones is far from complete. The evidence for ecdysteroid control of crustacean molting is impressive, but we still do not know which ecdysteroids are involved or how they regulate premolt development. There is uncertainty as to which ecdysteroid is taken up by the receptors in the cells of the target tissues, and whether ecdysone and 20-OH-ecdysone are both necessary for molting.

In vitro studies on locusts indicate that ecdysone and 20-OH-ecdysone have different roles in integumental morphogenesis: ecdysone brings about changes in epidermal cells prior to apolysis but does not induce cuticle deposition, whereas 20-OH-ecdysone induces apolysis and cuticle deposition. This apparent division of responsibility is supported by what is known about the relative titers of the two ecdysteroids during the molt cycle. Ecdysone is the primary ecdysteroid in insects prior to apolysis, but 20-OH-ecdysone predominates when the premolt cuticle is being deposited (Porcheron et al. 1984). In addition, it has been found that in vitro exocuticle formation in Drosophila requires a brief exposure to 20-OH-ecdysone, but that two sequential pulses may be necessary for exocuticle completion (Doctor et al. 1985).

In Orconectes, ecdysteroids are barely detectable immediately after ecdysis. Levels increase gradually in late stage A and plateau in stage B_2. There is another increase at stage D_0, coinciding with apolysis and initial gastrolith deposition, but the developmental processes of D_0 seem to require relatively small quantities of ecdysteroid. As in insects, the major premolt ecdysteroid peak occurs during stages D_1''' and D_2, at the time the preexuvial cuticle is deposited.

At this point in premolt, the ecdysteroid titer gradually decreases until shortly before ecdysis, then it abruptly declines to the low levels characteristic of stage A (O'Connor 1985; Stevenson et al. 1979; Willig and Keller 1973). This suggests a continuous but gradually decreasing biosynthesis of ecdysteroids during premolt. However, Stevenson et al. (1979) concluded ecdysone synthesis actually occurs as a series of pulses, a mechanism that might permit more precise control of the various premolt developmental processes.

The variation in type and quantity of ecdysteroid production noted in crustaceans and insects suggests a high degree of regulatory sophistication and coordination, since there are at least three distinct levels of ecdysteroid control: rate of ecdysone synthesis, type of ecdysteroid metabolism and rate of ecdysteroid inactivation and excretion.

If Y organ biosynthesis of ecdysteroids is tuned to the physiological changes of premolt, the regulatory mechanism must be reasonably responsive. Chang (1985) ascribed Y organ secretory regulation primarily to the X organ-sinus gland complex, but he recognized that other regulatory mechanisms must exist. The relative competence with which the new integument is formed in eyestalkless animals certainly confirms this. It is not known what these alternative mechanisms might be, but the secondary regulators of insect molt gland biosynthetic activity noted by Watson et al. (1985) are of interest.

Significant alterations in the pathways of ecdysteroid metabolism during the molt cycle have been noted by McCarthy (1982). Ecdysone produced by the Y organ is hydroxylated to several different products, including 20-OH-ecdysone. Although the 20-OH-ecdysone metabolic pathway predominates in all molt stages, it is virtually the only one of any significance during premolt.

Finally, there are large molt stage differences in the rate of turnover of ecdysone metabolites, a phenomenon noted in both insects and crustaceans. Crustaceans eliminate ecdysteroids by forming highly polar products in organs such as the non-vitellogenic ovary, hindgut and epidermis. In Carcinus, inactivation results in the formation of highly polar products and ecdysonoic acids (Lachaise and Lafont 1984). The inactivation mechanism is capable of effecting dramatic rate changes, producing a 10-fold increase in the rate of ecdysteroid elimination as the animal approaches molt (McCarthy 1982).

Molt-Accelerating Hormone

According to the classical explanation, crustaceans enter premolt after the inhibitory influence of eyestalk MIH is removed. However, Bollenbacher et al. (1972) determined that metabolic changes of early premolt are mediated by substances from something other than the eyestalk or molting gland, and there is considerable historical evidence for CNS enhancement of Y organ activity. Thus the postulation of a molt accelerating hormone (Vernet 1976, for review).

Exuviation Factor

The titer of molting hormone normally decreases before ecdysis but, if exogenous ecdysteroids are

used to maintain the titer at abnormally high levels, some species are unable to molt. There are a number of potential explanations for this phenomenon, but one that has received some attention is the exuviation factor first proposed by Graf (1972a, b) as a crustacean analog of the lepidopteran eclosion hormone.

The exuviation factor presumably is released from the Y organ when the ecdysteroid titer drops just before molt and is therefore blocked if exogenous ecdysteroids are administered. Graf developed the concept from work with amphipods, but it was extended to shrimp and crabs (decapods) by Touir and Charniaux-Cotton (1974) and Charmantier-Daures and Vernet (1974). To our knowledge, no one has yet reported the exuviation factor in crayfish.

Mandibular Organs

The mandibular organs of decapods have been a functional enigma since their discovery and subsequent distinction from the Y organ (Aiken 1980 for review), and experiments to elucidate their role in molting and growth have been inconclusive. Maturing American lobsters deprived of mandibular organs maintained normal molting rhythms for more than 2 yr (Byard et al. 1975), suggesting no involvement in the molting cycle of this animal, but Yudin et al. (1980) were able to reduce the intermolt time of shrimp with implants of blue crab mandibular organs (however, the corroborating crab study that they cited - Connell 1970 - is not as reported).

On the other hand, there have been reports suggestive of a role in reproduction: the mandibular organs of the American lobster reportedly contain quantities of progesterone and estradiol that vary with changes in ovary development (Couch and Hagino 1983), and mandibular organ implants appear to stimulate vitellogenesis in spider crabs (Hinsch 1980).

The ultrastructure of mandibular organs is similar to that of lipid or steroid-producing glands (Byard et al. 1975; Hinsch 1977, 1981; Le Roux 1968; Yudin et al. 1980), and an analogy with the insect corpus allatum was therefore suggested (Byard et al. 1975). Significantly, recent work by Laufer et al. (1987) indicates the mandibular organs of crabs and lobsters secrete methyl farnesoate (MF), a compound with juvenile hormone activity in insects, and that changes in MF concentration correlate with vitellogenic activity in the ovary. It now appears that the mandibular organs may be producing either a crustacean JH or a prohormone that is converted to some JH-active compound in peripheral tissues.

MANIPULATION OF MOLTING AND GROWTH

Techniques for inducing molt are of interest because soft-shelled crayfish command premium prices as bait and human food. Although a crayfish completes the soft-shelled stage in about 12 h, the 'paper-shell' stage lasts 36-48 h. Paper-shells as well as late-premolt 'busters' with the shell removed are sold as soft-shell crayfish (Avault and Huner 1985). Soft-shell crayfish can be produced by simply holding premolt crayfish in tanks until they molt, or by subjecting them to the types of surgical, environmental and hormonal manipulations discussed in the following sections.

Hormone Manipulation

Ecdysteroids have been administered to crustaceans by topical application, crystal implantation, injection or bath (Skinner 1985b; Vernet 1976, for review), but these treatments have yielded mixed results.

Of the ecdysteroids that have been used, 20-OH-ecdysone is the most active. When injected into crayfish, it is generally effective at doses from 0.5-5.0 µg/body weight (Krishnakumaran and Schneiderman 1970), although the effective dose can vary with season, reproductive state, molt stage and temperature (Aiken and Waddy 1975; Skinner 1985b). Injected ecdysteroids can produce everything from a protracted intermolt to an accelerated premolt that culminates in death at ecdysis (Aiken and Waddy 1975; Heyman 1971; Huner and Avault 1977; Krishnakumaran and Schneiderman 1968, 1970; Lowe et al. 1968).

As noted previously, 20-OH-ecdysone seems to be more involved in the stimulation of setal development and cuticle synthesis rather than the induction of premolt, and a dose that is adequate to overcome endogenous molt inhibition and induce premolt during a non-molting period will often cause abnormal calcium metabolism and excessive and abnormal integumentary development (Aiken 1980; Krishnakumaran and Schneiderman 1970). The lethal effects can usually be avoided by using lower doses preceded by eyestalk ablation, by using a slow release form of the hormone or by pretreating with ecdysone (Aiken 1980, for references).

Results obtained with Procambarus have been similar to those from other crustaceans. Although there have been some contradictory studies (cf. Lowe et al. 1968; Krishnakumaran and Schneiderman 1969, 1970), these may simply reflect seasonal variability.

There have also been practical attempts to induce molting in Procambarus and Orconectes with the ecdysteroids ponasterone A, inokosterone, ecdysone and 20-OH-ecdysone. Heyman (1971) advised that dose and molt stage be carefully matched and he reported a 99% yield of soft-shelled crayfish from ecdysteroid treatment. Unfortunately, specific methods were not given. Huner and Avault (1977), encouraged by Heyman's results, injected P. clarkii with 3 µg/g body weight of 20-OH-ecdysone in an attempt to produce soft-shelled crayfish but all died at molt.

The administration of ecdysteroids via diets or dilute baths was rejected by Heyman (1971) as unsatisfactory, but work with isopods suggests that hormone baths can be effective if the timing of exposure is carefully matched to molt stage (Maissiat and Graf 1973).

Molt mortality is a common phenomenon in studies utilizing exogenous ecdysteroids. The treated animal undergoes accelerated premolt but dies while attempting to cast off its old shell. This suggests an abnormality in the sequence of physiological events that lead to ecdysis, probably induced by type, timing or dose of exogenous hormone.

Several studies have indicated a seasonal fluctuation in the molt-death phenomenon. Molt mortality is much more common when the animals are injected with effective levels of ecdysteroid during non-molting seasons. Interestingly, earlier studies showed that photoperiod manipulations affected northern Orconectes virilis in a similar way. This prompted the suggestion that MIH titer might be regulated by photoperiod in this species (Aiken 1969). It would be interesting to combine the effects of long photoperiod and ecdysteroid injection to see whether the mitigating effects of eyestalk ablation could be duplicated. This would provide insight into the control of MIH as well as control by MIH.

Eyestalk Ablation

When the eyestalks with their X organ-sinus gland neurosecretory complex are removed from a decapod, the processes leading to molt are induced and rapidly completed. However, as Brown and Cunningham (1939) discovered, eyestalk ablation can also produce a debilitating and frequently lethal hormonal imbalance.

This is because eyestalk ablation removes not only MIH but at least five other neurosecretory hormones that are involved in regeneration of limbs, development of gonads, organization of cuticle and control of chromatophores, retinal pigment migrations, hemolymph sugar levels and ionic and osmotic homeostasis (Green and Neff 1972; Spaziani et al. 1982).

This degree of metabolic disruption would be expected to produce heavy mortality and this has often, but not always, been the case (Bittner and Kopanda 1973; Brown and Cunningham 1939; Huner and Avault 1977; Huner and Lindqvist 1984; Nakatani and Otsu 1979; Smith 1940). Juveniles appear more likely than adults to survive a molt following eyestalk removal (Avault and Huner 1985; Huner and Avault 1977), and it has been said that mature male Astacus astacus seldom survive (Huner and Lindqvist 1984). In the American lobster, a seasonal sensitivity to eyestalk ablation has been detected, especially in males. Those ablated in autumn survive well, whereas those ablated in spring do not (Aiken 1980). In juvenile American lobsters, unilateral eyestalk ablation accelerates molting and growth without the mortality associated with bilateral ablation (Peutz et al. 1987).

Eyestalk-ablated lobsters that survive the first few postoperative days generally survive the molt as well, but reports indicate eyestalk-ablated crayfish often die at molt. There are also references to eyestalkless crayfish that completed one molt but died at the second molt, and eyestalkless crayfish that were hollow-tailed (very little meat) after completing two or three molts (Huner and Avault 1977). Both the molt-death and the hollow-tail scenario could result from a nutritional problem but, to our knowledge, that aspect has not been investigated in crayfish. In any event, death at molt in cultured crayfish need not be a total loss since hand-peeled 'busters' command the same price as newly molted crayfish (Avault and Huner 1985).

The dramatic shortening of the molt cycle that follows eyestalk ablation is due to the immediate entry of the animals into premolt and a significant reduction in the time required to complete premolt. Chang and Bruce (1980) evaluated changes in circulating ecdysteroid titer and suggested the time between molts was reduced due to the elimination of molt stages in which circulating ecdysteroids are at a basal level (i.e. stages A, B and C).

Eyestalk ablation may cause circulating ecdysteroid titer to increase more than in a normal molt cycle (Jegla et al. 1983; O'Connor 1985), an increase that is consistent with the higher rate of ecdysteroid secretion by the Y organ of eyestalkless animals. Several days before ecdysis the ecdysteroids of eyestalkless crabs may be eight times greater than in intact control crabs (O'Connor 1985). The situation in Orconectes limosus is different in that there is a more gradual effect of eyestalk ablation on ecdysteroid titer (Jegla et al. 1983).

Size increase at molt in destalked Orconectes can be three times that in intact controls, probably as a consequence of increased water uptake at molt (Scudamore 1947). In Procambarus, the intermolt time of destalked animals was decreased to 25% and percent linear increase was doubled, producing a growth rate six times greater in destalked animals than in intact controls (Nakatani and Otsu 1979).

Limb Loss

Limb autotomy occurs when a crustacean breaks off its own appendage at a preformed breaking point close to the body. No muscles cross the breakage plane, so there is minimal tissue damage (Skinner 1985b) and the autotomized appendage soon regenerates. For many crustaceans the loss of a critical number of appendages is a stimulus to enter premolt, undergo regenerative growth of the missing appendages and molt. Limb removal is therefore a way to induce precocious molts without encountering the many problems of eyestalk ablation.

The responses of different species to limb injury are variable. The fiddler crab readily autotomizes injured appendages, but the crayfish Procambarus is often reluctant to autotomize in response to injury and more drastic stimuli are required (Bittner and Kopanda 1973). Once autotomy is induced, the molt frequency of Procambarus will be increased (Bittner and Kopanda 1973) but linear increase at molt may be reduced (Nakatani and Otsu 1981).

In addition to the variable responses between species, factors such as age, sex, molt stage and maturity can affect the autotomy response within a species. Skinner (1985a, b) summarized available information on regeneration and its effect on the molt cycle, and explained some of the variable results that have been reported.

In Gecarcinus, at least five appendages must be lost before premolt will be induced but, in many crustaceans, the energy demands of massive regeneration will cause a delay rather than an acceleration of molt. Once Gecarcinus reach stage D_0, the loss of one limb can cause premolt to be delayed until the regenerating limb has reached the size of those that regenerated earlier but, if the loss occurs at stage D_1 or later, regeneration will be postponed until the next cycle and there will be no effect on the immediate premolt.

The nature of the molt-inductive stimulus that follows limb loss is not known, but it is thought

that the Y organs are stimulated to release molting hormone. The stimulus is not due merely to loss of tissue mass (Skinner 1985b), and simply cutting the leg nerves will accelerate molt frequency in Procambarus (Bittner and Kopanda 1973).

Multiple autotomy is not as effective as eyestalk ablation at accelerating premolt or promoting size increase at molt, but it has the advantage of causing much less physiological disruption and mortality. If the objective of manipulation is simply to produce a molt without regard for growth, multiple autotomy is worth considering.

Environmental Control

Production of soft-shelled crayfish in intensive culture can be enhanced by using deionized water in molting tanks. Crayfish will molt successfully in deionized water but will be unable to harden completely.

Methods for retarding growth of P. clarkii may be necessary for culturing them as bait (Huner and Avault 1976b). As growth of P. clarkii is dependent on hydrological conditions, water levels in ponds can be manipulated to alter growth rates. P. clarkii burrow and hatch their young during the dry season (June-August) and there is little growth of the young in the burrows. When the ponds are flooded, the young are flushed out of the burrow and begin to forage and grow. Therefore, the growth rates of young crayfish in different ponds can be staggered by simply flooding the ponds in sequence. In this way, the young crayfish will reach the preferred bait size (50-70 mm) between early October and the end of May when ponds are again drained (Huner and Avault 1976b).

SUMMARY

Crayfish increase their physical size by periodically shedding their calcified external skeleton and expanding their flexible underlying integument. Their rate of growth thus consists of two separate but related components: frequency of molting and size increase at molt. To grow rapidly a crayfish must molt frequently and increase its size significantly at each molt.

Photoperiod, temperature, nutrition, hydrological conditions, stress and reproductive state are a few of the many things that can affect molting frequency. Size increase at molt can also be altered by many of these same factors, but usually in a different way or at a different time. For example, inadequate nutrition after molt may prolong tissue growth and delay the onset of the next molt but, if premolt has already been induced, inadequate nutrition is not as likely to delay the molt as to reduce the size increase at molt.

The ability of crayfish to molt and increase in size is compromised by many factors in the environment, but temperature is perhaps the dominant influence. At the northern extreme of North American distribution, the temperature may be too low for molting in all but two or three months during the summer. At the southern extreme, on the other hand, the temperature may be too high for molting during the summer months, and the animal must molt and grow during fall, winter and spring.

The crayfish integument consists of a cellular epidermis overlain by four distinct layers of cuticle. Two of these cuticular layers are formed before the molt and the other two are added after the molt. The two layers of the flexible premolt cuticle therefore underlie the inflexible layers of the old, heavily calcified exoskeleton and, when the latter is cast off at molt, this premolt cuticle becomes the new exoskeleton, complete with muscle and nerve attachments and direct communication with the cells of the underlying epidermis. The epidermis then synthesizes the final two postmolt layers and mineralizes the new exoskeleton.

In preparing for a molt, a crayfish undergoes complex biochemical and morphological changes. The epidermis separates from the old cuticle and begins secreting enzymes that dissolve the inner layers and remove mineral for storage. Some of this stored mineral is used after the molt to harden critical parts of important appendages - mouthparts, chelipeds, walking legs and other components that will enable the postmolt crayfish to begin foraging.

The large chelipeds create special problems for the molting crayfish because the muscle of the large claw is too massive to pass through the thin upper segments of the leg. To make this passage possible, the claw muscle is degraded to a fraction of its intermolt volume and the upper leg segments are partially decalcified. At molt, the atrophied muscle is easily withdrawn through the decalcified and expanded upper leg segments.

An additional difficulty is caused by the relationship between the premolt cuticle and the rigid exoskeleton. An animal molts so it can increase its size, but the premolt cuticle is secreted beneath the exoskeleton, which means the volume of the premolt body encased within its new cuticle is less than that of the old body it is about to replace. The ingenious solution involves two mechanisms. First, the premolt epidermis with its flexible cuticle undergoes rapid growth just before molt, forming expansion folds and ripples beneath the rigid exoskeleton. Second, water is rapidly ingested and absorbed by the newly molted animal, inflating and expanding the soft integument. The molted crayfish is therefore able to become longer by some 10-15% and heavier by 30-50%, although this increased volume is largely water that will have to be replaced by tissue growth in ensuing days.

All of the complex processes involved in molt preparation and recovery are controlled by the endocrine system. Environmental information is translated into hormonal stimuli which are orchestrated to produce the appropriate effect on the animal. A simplistic view holds that molting is controlled by two hormones or groups of hormones: molt inhibiting hormone (MIH), that suppresses molting, and molting hormone (MH), that induces premolt and regulates the processes that culminate in molting. MIH is a protein that is synthesized and released by the eyestalk neurosecretory complex. It is thought to suppress molting activity by preventing the so-called Y organs from synthesizing and releasing MH, a collective term for one or more ecdysteroids that have molting hormone activity in a variety of arthropods.

Stressful conditions can interfere with the induction or completion of some of the many steps

involved, and cause a flawed or fatal molt. Given the complexity of the molting process and the demands that it places on a crustacean, it is remarkable that problems occur as infrequently as they do.

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